New routes to chiral Evans' auxiliaries by enzymatic desymmetrisation and resolution

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Submitted by Claudia Neri

for the degree of PhD

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Finally I would like to thank my family and Diego for playing the key part in the last three years.

Abstract

This report describes how enantiomerically enriched Evans' auxiliaries can be successfully prepared by either an enzymatic desymmetrisation strategy or an asymmetric synthesis using racemic auxiliaries and an enzymatic resolution.

Desymmetrisation of *N*-Boc-protected serinol has been achieved in good yield and high enantiomeric excess using porcine pancreas lipase. This has been exploited in different ways to prepare enantiomerically enriched (4*R*)- and (4*S*)-substituted 2-oxazolidinones.

Starting from racemic Evans' auxiliary, by means of a diastereoselective aldol reaction coupled with a lipase-catalysed resolution, we achieved the preparation of enantiomerically enriched β–hydroxy acids and enantiomerically enriched 2-oxazolidinones.
Our methodology allows the use of not only racemic amino acids but also racemic amino alcohols to achieve asymmetric synthesis. It also offers, for the first time in chiral auxiliary-based chemistry, the possibility of using designed racemic auxiliaries, instead of the difficult to prepare enantiomerically pure equivalents. In addition the procedure acts as an indirect enzyme-catalysed resolution of Evans' auxiliaries.

Enzymatic resolution of racemic Evans' auxiliaries and their *N*-acyl equivalents has been attempted.

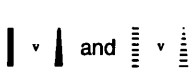
Racemic *N*-acylated-5-substituted-2-oxazolidinone was synthesised using a one-pot synthesis from a carbamate and a monosubstituted epoxide, but the reaction proceeded in low yield. Nevertheless, we have demonstrated that there is a scope for successful future attempts with some variations for the one-pot synthesis of *N*-acylated-4,5-disubstituted-2-oxazolidinones.

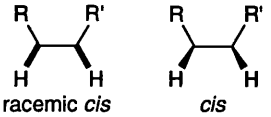
Abbreviations

Ac	Acetyl
ANL	*Aspergillus niger* lipase
app.	Apparent
Aux	Auxiliary
Bn	Benzyl
Boc	*N*-*tert*-Butoxycarbonyl
br	Broad
Bu	Butyl
c	Concentration
CAL B	*Candida antarctica* lipase type B
CCL	*Candida cylindracea* lipase
CLEC	Cross-Linked Enzyme Crystals
conv.	Conversion
CRL	*Candida rugosa* lipase
D	Diastereomeric ratio
DABCO	1,4-Diazabicyclo[2.2.2]octane
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
d.e.	Diastereomeric excess
DMAP	4,*N*,*N*-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
E	Enantiomeric ratio
e.e.	Enantiomeric excess
EP	Ethyl propionate
Et	Ethyl
FC	Flash Chromatography
h	Hour
hepes	4-(2-Hydroxyethyl)piperazine-1-ethansulfonic acid
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
i	*Iso*
IPA	Isopropyl alcohol
^{i}Pr	*iso*-Propyl

IR	Infra-Red
J	Coupling constant
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazide
M	Molar
Me	Methyl
m	*Meta*
min	Minutes
MJL	*Mucor javanicus* lipase
MS	Mass Spectrometry
n	*Normal*
NMP	*N*-Methyl-2-pyrrolidinone
NMR	Nuclear Magnetic Resonance
p	*Para*
PCL	*Pseudomonas cepacia* lipase
PFL	*Pseudomonas fluorescens* lipase
Ph	Phenyl
PPL	Porcine pancreas lipase
ppm	Parts per million
Pr	Propyl
PSL	*Pseudomonas* species lipase
R	Unspecified alkyl group
rt	Room temperature
sat.	Saturated
t	Time
t	*tert*
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine
tris	Tris-(hydroxymethyl)aminomethane
VA	Vinyl acetate
VP	Vinyl propionate

Note:





1. Lipases as Biocatalysts in Organic Synthesis

1.1 Introduction

Enzymes' role in Nature is to catalyse and coordinate a multitude of chemical reactions necessary to life. Chemical reactions, without the presence of enzymes, would be too slow to be effective under the conditions prevalent in normal living systems (aqueous environment with pH 7 and temperatures between 20 and 40 °C). Since the end of the 19th century, it has been known that enzymes do not require the environment of a living cell to be active. This opened the way to many applications in food technology, in the production of leather, textiles and paper, in diagnostics and food analysis and finally in the production of chemicals by biotransformations.
Enzymes may be chosen as catalysts for one or more of the following reasons:

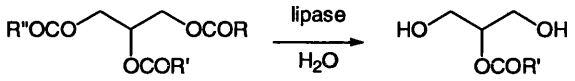
- high selectivity in complex mixtures;
- high stereo- and regioselectivity of reactions;
- absence of side reactions leading to higher yields and simpler separation processes ;
- savings in energy costs owing to mild reaction conditions.

Of course, enzymes have limitations, as any other highly specialised catalysts. The most notable consequence arises from the selectivity of enzymes with respect to the substrate bound and the type of reaction catalysed. Such high selectivity means that that it may be difficult to cover the variety of chemical reactions desired in organic synthesis. The enzyme needed in a particular case may not be readily available. However, there are many enzymes discovered all the time and an increasing number can be obtained commercially.

1.2 Lipases. General applications

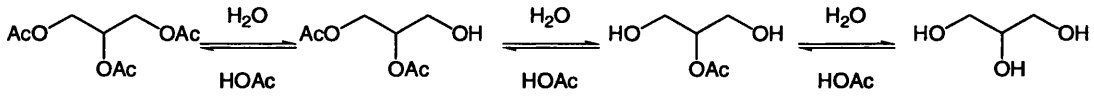
Lipids are key elements in the biological chemistry. Most organisms use the supramolecular chemistry of phospholipids to form their exterior and compartmental membranes. Many plants and animals store chemical energy in the form of

triglycerides, which are poorly soluble in water. For the metabolism of these and other biochemicals, they produce hydrolases, enzymes that can hydrolyse bonds of water-soluble esters. Lipases are, more systematically, triacylglycerolacyl hydrolases (E.C.3.1.1.3), which can hydrolyse triglycerides at the water/oil interface. Many lipases exhibit 1,3-specificity, as illustrated in Scheme 1.1.



Scheme 1.1. Lipase-catalysed hydrolysis of triglycerides

Although the natural role of lipases is the degradation of food and fat and their use as useful drugs against digestive disorders and diseases, the application of lipases in biotechnology (as detergent additives) and as catalysts for the manufacture of specialty (oleo)chemicals and for organic synthesis has been widely exploited.[1]
Their broad synthetic application is mainly due to the fact that lipases, if compared to other enzymes, are very versatile, and thus, changing the solvent system, they can be used to perform hydrolysis reactions or ester syntheses (Scheme1. 2).



Scheme 1.2. General hydrolysis and ester synthesis with lipases

In addition, lipases can accommodate a wide range of substrates other than triglycerides as aliphatic, alicyclic, bicyclic and aromatic esters.[2,3] With respect to racemic esters or substrates with several hydroxyl groups, lipases also react with high enantio- and regioselectivity.[4]
Finally, the acyl enzyme complex in lipase-catalysed reactions can be formed not only from carboxylic esters but also from a wide range of other substrates such as thioesters or activated amines and amides, which expands the synthetic utility of lipases considerably.[5-9]
As a result, today chemists may choose their preferred lipase from a great number of commercially available preparations or even explore a range of different lipases and esterases marketed as a kit.

1.3 Lipase origins and preparation

Lipases are widely distributed amongst animals, plants and microorganisms. They are usually obtained by either extraction from animal or plant tissue or by fermentation by microorganisms. Commercially available lipases are usually derived from microorganisms. One of the reasons why lipases are so popular enzymes in organic synthesis is that lipases are extracellular enzymes, which are secreted into liquid and solid culture and are relatively stable in cultivation media. This makes them easy and inexpensive to extract.

Last generation lipases are from recombinant bacterial and yeast. Produced by genetic engineering techniques, many of them are today commercially available. For example, the "detergent lipase" from fungus *Humicula lanuginosa* is commercially produced in large scale (several 100 tons per year) through fermentation of *Aspergyllus oryzae* into which the gene coding for *Humicula lanuginosa* lipase was cloned.[10]

The molecular weight of the known lipases in their active, native form ranges from 30 to 65 kDa. Lipases are generally soluble in water and insoluble in organic solvents, and may be strongly adsorbed at the air/water interface.

Usually, commercially available lipases are a mixture of proteins that may contain other hydrolases together with stabilizing supports. Pig pancreas lipase is, for example, a glycoprotein, which exists as a mixture of isoenzymes differing in the glycan moiety of the enzyme. Crude pancreas lipase contains presumably another carboxyl esterase that may be responsible at least in part for the high enantioselectivity frequently observed with this enzyme in hydrolysis and esterification.[11-14]

Therefore, an isolated lipase of the same origin may have different activities and selectivities depending on the isolation and purification procedures used by the individual suppliers. The lipase of interest can be separated from other esterases and proteases occurring in the crude enzyme preparation. However, purification protocols are often long and complex, because the affinity of lipases is high not only at the oil/water boundary, but to any interface of lower polarity than water (e.g. water-immiscible organic solvents, glass and plastic surfaces and air bubbles); lipases may irreversibly adsorb and denature at such interfaces.[15-19]

Table 1.1 summarises commercially available lipases most frequently used in organic synthesis and their abbreviation codes.

Table 1.1. Commercially available lipases most used in organic synthesis

ORIGIN	CODE
Mammalian origin	
Porcine pancreas lipase	PPL
Fungal origin	
Candida rugosa or *Candida cylindracea*	CRL or CCL[a]
Candida antarctica type B	CAL B
Candida antarctica type A	CAL A
Mucor miehei or *Rhizomucor miehei*	MML or RML[a]
Bacterial origin	
Pseudomonas species	PSL
Pseudomonas fluorescens or *Pseudomonas cepacia*	PFL or PCL[a]

[a] Some microbial lipases, used in the past with a name, were recently re-identified with regard to their origin and name.

1.4 Lipase structure and mechanism

The first definition of a lipase is dated 1958. It was given by Sarda and Desnuelle[20] in kinetic terms and was related to the phenomenon of interfacial activation. This derived from the observation that lipase activity is low on monomeric substrates but strongly enhanced once an aggregate "supersubstrate" (such an emulsion or a micellar solution) is formed above its saturation limit. For a long time lipases were considered as a special class of hydrolases, which are highly efficient at hydrolysing molecules aggregated in water.

Only in 1990 the two first lipase structures were solved by X-ray crystallography. A unique mechanism of catalysis, unlike that of any other enzyme, was revealed. These three dimensional structures suggested that interfacial activation might be due to the presence of an amphiphilic peptidic loop covering the enzyme active site in solution, just like a lid or a flap.[21,22] From the X-ray structure of cocrystals between lipases and substrate analogues, it was evident that when contact occurs with a lipid/water interface, this lid undergoes a conformational rearrangement which makes

the active site accessible to the substrate.[23] The position of the flap and the active site in *Candida rugosa* lipase is shown in Figure 1.1.

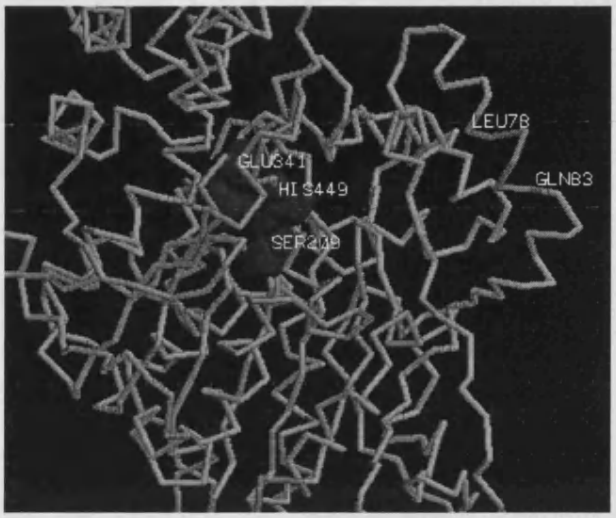
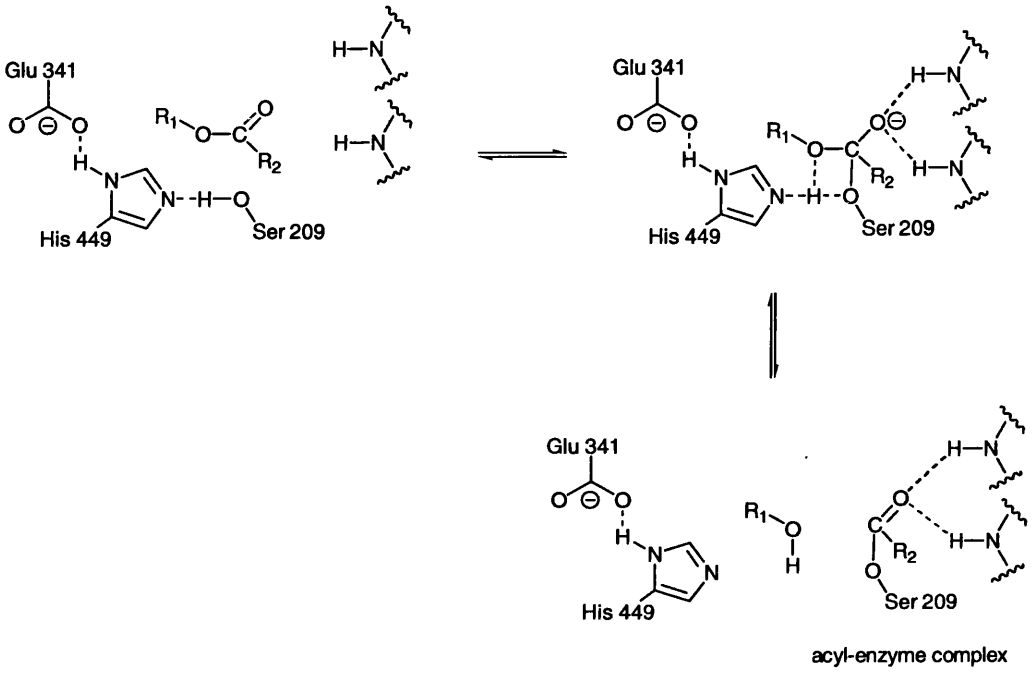


Fig. 1.1. Lipase from *Candida rugosa* active site (SER 209, GLU 341, HIS 449) and flap (LEU 78 and GLN 83)

However, subsequent studies provided evidence that not all lipases subscribe to the phenomenon of interfacial activation. Thus, *Candida antarctica* type B, whose tertiary structure is known, has an amphiphilic lid covering the active site, but does not show interfacial activation.[24] Among the pancreatic lipases whose tertiary structures have been solved, human pancreatic lipase contains a lid with 23 amino acids,[25] whereas coypu pancreatic lipase does not, although it has a lid of homologous size.[26] As a consequence, neither the phenomenon of interfacial activation nor the presence of a lid domain is appropriate criteria to classify an hydrolase into the lipase subfamily. The best experimental evidence remains whether or not it can catalyse the hydrolysis of long-chain acyl glycerols.

As can be seen in Fig. 1.1, the active site of lipase is in the form of a catalyic triad, composed of a nucleophilic serine residue activated by a hydrogen bond in relay with histidine and aspartate or glutamate (Scheme 1.3). Crucial to the catalytic function of the enzyme are, beside the interplay of the residues of these amino acids, the stabilization of the oxy anion intermediates and the corresponding transition states. The stabilisation occurs through hydrogen bonds provided by amide bonds or other

amino acid residues of the active site. Serine proteases follow essentially the same mechanism.



Scheme 1.3. Catalytic mechanism of lipase based on the catalytic triad. The numbering of amino acids refers to lipase from *Candida rugosa*

In summary, all lipases investigated so far exhibit a surprising degree of structural and functional similarity, regardless of the organism from which they are isolated and even if the observed amino acid sequence homologies are low.[27,28] However, variations in the architecture of the substrate binding site may have a strong effect on the catalytic properties, the temperature and the stability of a lipase in a solvent.

1.5 Lipase immobilisation

Despite the potentiality of lipases as catalysts for the synthesis of a variety of intermediates, the practical application of enzymes as catalysts in organic chemistry is often limited by the inherent differences between the conditions in which lipases catalyse biological reactions and the conditions required in a preparative chemical synthesis. Moreover, while biological systems destroy and regenerate enzymes as they are needed, catalysts used in chemical manufacture must be often recovered and recycled many times for economical reasons.

The most commonly used strategy to give the desirable features of conventional heterogeneous catalysts to biological catalysts is immobilisation.
By definition, enzyme immobilisation is the conversion of an enzyme to a form with restricted mobility but with retention of its catalytic function.[29] This limited mobility is generally achieved by either conversion to an insoluble form (for example by attaching the enzyme to insoluble particles) or by containment within a semi-permeable barrier (for example entrapment within a membrane). During this immobilisation process, enzymes acquire advantageous properties:

- Immobilised enzymes can be recycled.
- They can be used continuously.
- They can be easily separated from soluble reaction products and unreacted substrate, thus simplifying work-up and catalyst recovery.
- Catalytic properties and enzyme stability can be increased in the immobilised form.
- The immobilised preparation prevent microbial contamination usually found in the soluble protein.

Of course, there are some limitations on the utility of immobilised enzymes. First, the carrier can be quite expensive and its cost may even exceed the cost of the enzyme itself. Second, the activity of the resulting immobilised enzyme is often reduced because of the chemical modification of the protein and mass transfer limitation. Finally, the yield of the protein binding is hardly quantitative and this dramatically reduces catalytic activity per weight of solid.
Despite these limitations, many commercially available lipases are actually immobilised. The immobilisation on solid supports can be achieved in different ways; The technique used can be a non-covalent adsorption, covalent attachment, entrapment of the lipase in a polymeric gel, membrane or capsule, cross-linking with a polyfunctional agent.
For example, lipases from *Candida antarctica*, *Humicola lanuginosa* and *Mucor miehei* have been immobilised by ionic attachment to synthetic resins. Methacrylate resin crossed-linked with divinylbenzene have been employed for the transesterification of fats and oils, affording virtually quantitative binding of the protein. The preparation of *Candida antarctica* lipase B has been widely used for the resolution of carboxylic acids and alcohols.[30] A variety of immobilised *Pseudomonas*

cepacia lipase preparations have also been compared for their activity and selectivity in organic solvents.[31]

More recently, cross-linked enzyme crystals (CLEC®) have been shown to be highly active and stable heterogeneous biocatalyst preparations. In this method, protein microcrystals are produced from aqueous solution. The protein molecules in the microcrystals are then covalently cross-linked by treatment with an appropriate multi-functional reagent, usually glutaraldehyde. This renders the crystals insoluble on transfer to different aqueous media.[32] Reetz[33] and others[34,35] have found that entrapment of lipases within a hydrophobic silica sol-gel can result in a biocatalyst whose activity in organic media is enhanced in comparison to the corresponding lipase powder under the same conditions. A silica matrix is generated in the presence of an aqueous solution of the lipase by treating hydrophobic alkyl alkoxysilanes with a catalytic amount of sodium fluoride. The gel is allowed to set, then dried and crushed to the desired average particle size.

1.6 Lipases in organic solvent. Solvent engineering

The use of lipases in organic solvents has proved to be an advantageous alternative to reactions in water, especially for poor water-soluble substrates and products, e.g. in the synthesis of esters, lactones or selected peptides. The choice of organic solvent in a lipase-catalysed reaction is often crucial and can influence enzyme activity and selectivity. Even if the knowledge of how this happens is not yet available, there is much work about the solvent engineering of lipase-catalysed reactions.

Organic solvents can have both stabilising and destabilising effects on lipase activity. A reasonably good measure of the compatibility of solvents with enzymes is the log *P* value, where *P* is defined as the distribution coefficient of a solvent between water and 1-octanol in a two-phase system.[36] Solvents with a log *P* value above 4 are usually suitable (e.g. aromatic, aliphatics) whereas water-miscible solvents with a log *P* value below 2 (short chain esters, DMF, short-chain alcohols) are not suitable for employment with enzymes. These solvents with log $P<2$ seem to interfere with the water at the boundary of the protein itself and so with the binding forces necessary to maintain the enzyme in its active form.

Lipases can be used in *one-phase systems*, which may consist of water, water plus a water-miscible solvent or a pure organic solvent. Most water-miscible solvents can be used in concentrations up to 20% before enzyme deactivation occurs and the benefits obtained by better solubility of substrates and/or selectivity are lost. Using

this approach, enzyme activity and selectivity have to be examined carefully to select appropriate reaction conditions.

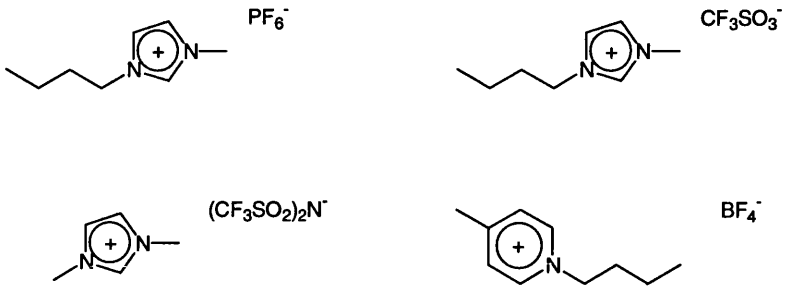
Several approaches can be employed to use mainly water non-miscible solvents. In most cases, the organic solvent has to be saturated with water in order to maintain the boundary water surrounding the enzyme and to avoid deactivation. The control of water activity can be achieved by addition of salts or utilization of saturated salt solutions.[37,38] The simplest way of using a lipase in organic solvent is to suspend the insoluble enzyme in the required solvent (straight from the bottle!). This technique has become general practice over the last few years.[39,40]

Supercritical carbon dioxide, which exhibits similar properties to hexane, can also be used as an alternative to organic solvents. This possesses a liquid like density (and thus high solvent strength) and gas like transport properties (low viscosity), which allow good mass transfer and facile extraction.[41]

To achieve true homogeneous catalysis, enzyme solubility may be increased by adding polyethylene glycol to its surface.[42] By use of detergents and small amounts of water or buffer, reversed micelles can also be formed. These contain the enzyme in the water phase while the organic solvent forms the bulk phase.[43,44]

Just like one-phase systems, *two-phase systems* may consist mainly of water or mainly of organic solvent. In the aqueous-based system an insoluble substrate is dispersed, using a non-miscible solvent (oil-in-water emulsion). A water-in-oil emulsion is obtained when the aqueous phase is dispersed in a water-immiscible solvent. Hydrolysis reactions of poorly water-soluble substrates (e.g. fat hydrolysis) may be performed in this way. Enzymes immobilised on an insoluble support also belong to two-phase systems.

Very recently ionic liquids have been introduced as a new class of solvents. These are salts with melting points below 100 °C and are non-volatile. Some typical structures are shown in Scheme 1.4. Many substrates and catalysts are soluble in ionic liquids[45,46] and they can be used to replace organic solvents in enzymatic reactions.[47-49] For example, the lipase-catalysed resolution of phenylethanol was carried out in a ionic liquid and an improved enantioselectivity at higher temperatures was observed compared to the selectivity obtained in methyl-*tert*-butylether (MTBE).[48,49]



Scheme 1.4. Typical structures of ionic liquids

1.7 Lipases in organic synthesis

Lipases are one of the most versatile classes of biocatalysts in organic synthesis. The application of lipases as biocatalysts has been reviewed in a number of books and journals.[50-53] Lipases are a unique class of hydrolases[54-56] for asymmetric synthesis based on prochiral and racemic substrates, for different important reasons:

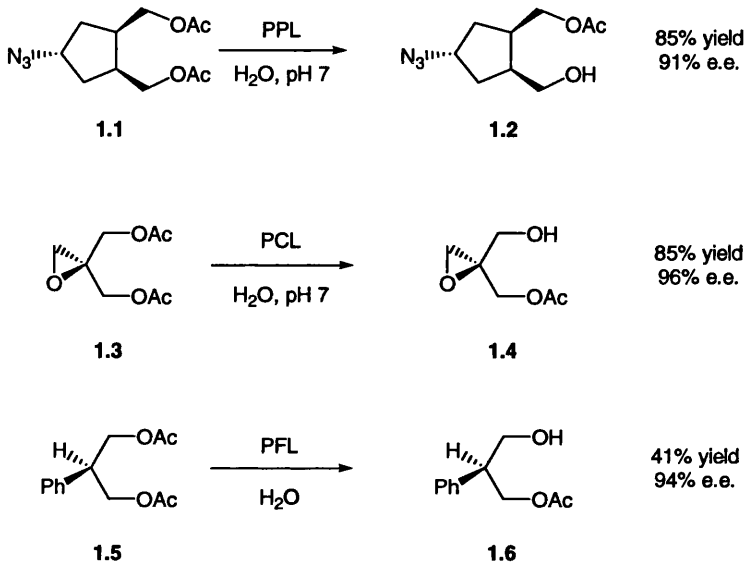
- ✓ Lipases accept non-natural substrates of enormous structural diversity. The wide substrate specificity of this enzyme class allows acylation of nucleophiles other than those with hydroxyl groups, while still showing regioselectivity or chiral recognition.
- ✓ Lipases act at the water/lipid boundary, which exhibits high interfacial energy. To withstand the denaturing effect of the interface, lipases have evolved unusually stable structures that may be active in water, in almost anhydrous organic solvents, and in supercritical fluids.[57-59]
- ✓ The free energy of lipase-catalysed fat hydrolysis is close to 0 $KJmol^{-1}$. As a result, thermodynamic equilibria are largely governed by reactant concentrations, and lipase-catalysed ester hydrolysis in water can easily be reversed into ester synthesis or transesterification, in non-aqueous media.

1.7.1 Lipase-catalysed reactions

1.7.1.1 Hydrolysis and formation of C-O bonds

Catalysis of the hydrolysis and formation of C-O bond if an ester, lactone or carbonate by lipases are among the most useful enzyme-catalysed reactions in organic synthesis.[2,3] The most important application is in the field of asymmetric synthesis.

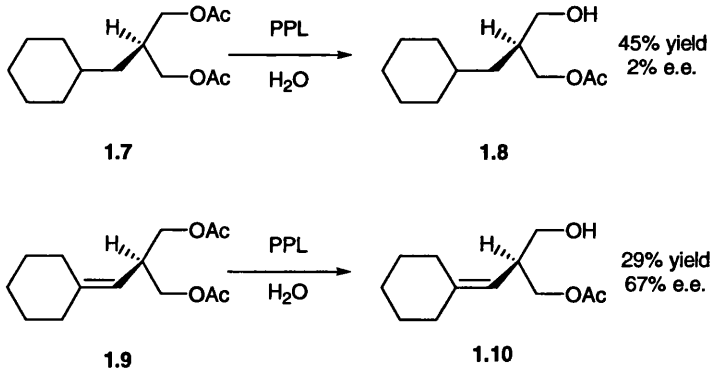
The first class of compounds that are appropriate substrates for lipase-catalysed reactions are principally those compounds which bear enantiotopic groups. These enantiotopic groups can be, for example, ester groups with the prochirality contained in the diol part of the molecule, like in diacetates **1.1**,[60] **1.3**[61,62] and **1.5**[63] (Scheme 1.5).



1.1 **1.2** **1.3** **1.4** **1.5** **1.6**

Scheme 1.5. Enantiotopos-differentiating lipase-catalysed hydrolysis of diacetates

Primary acyclic diacetates, such as compound **1.5** in Scheme 1.5, are substrates per excellence for lipases. Unsaturation in the alkyl chain frequently leads to the monoacetate of a higher e.e. value as exemplified with **1.7** and **1.9**[64] in Scheme 1.6.

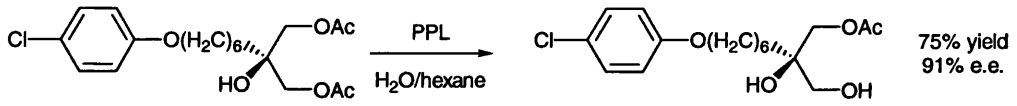


1.7 **1.8** **1.9** **1.10**

Scheme 1.6. Lipase-catalysed hydrolysis of saturated and analogous unsaturated diacetates

Comparison of the enantioselectivities of the hydrolysis of diacetates to the corresponding monoacetates is often complicated by the lack of information on the amount of diol formed. The latter arises from the hydrolysis of the monoacetate that may proceed under enantiomer differentiation, and thus the e.e. value of the monoacetate will be a composite of two enantioselective processes.

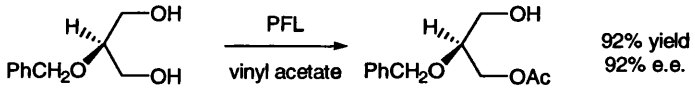
Glycerol diacetate derivative **1.11**, with a substituent in the 2-position, is hydrolysed with crude pig pancreas lipase in a two-phase system composed of water and hexane to the monoacetate **1.12** with good enantioselectivity (Scheme 1.7).[65] Hydrolysis in aqueous solution alone is much less selective.



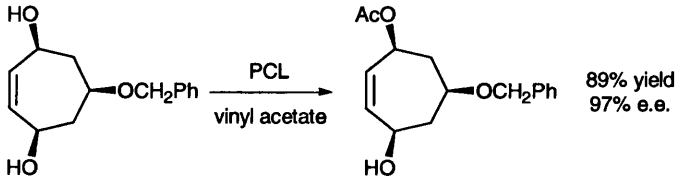
1.11 **1.12**

Scheme 1.7. Lipase-catalysed hydrolysis of diacetate **1.11** in a water/hexane system

Alternatively, lipase substrates can be compounds that carry enantiotopic hydroxyl groups, such as diols **1.13**[66] and **1.15**[67] (Scheme 1.8).



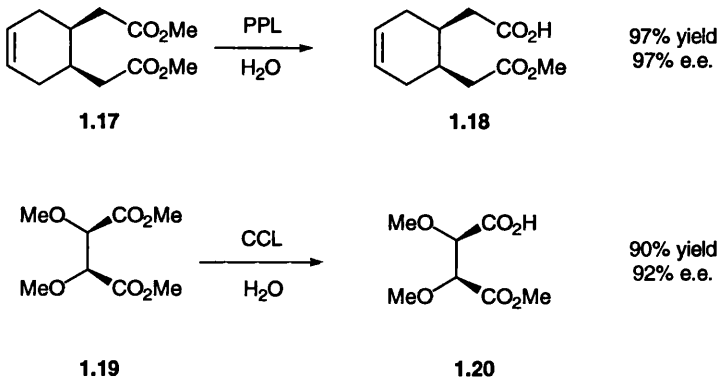
1.13 **1.14**



1.15 **1.16**

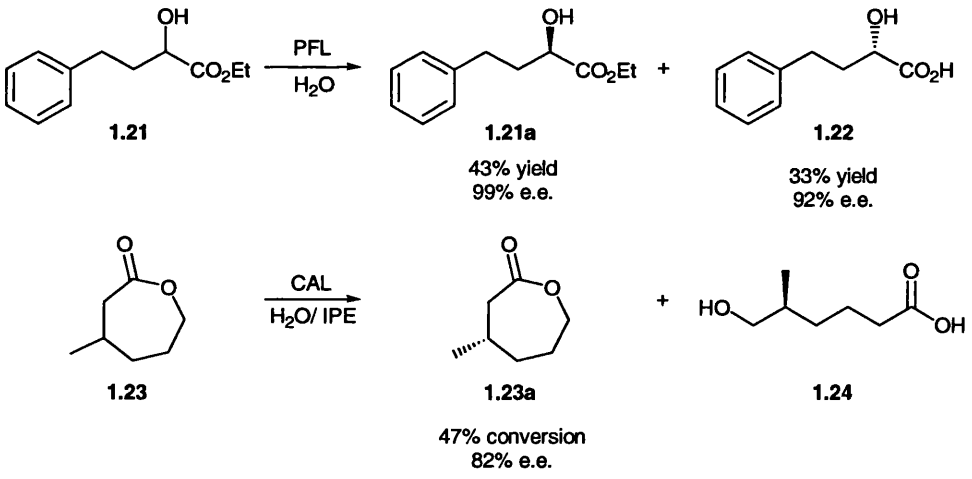
Scheme 1.8. Enantiotopic-differentiating lipase-catalysed transesterification of diols

A limited number of acyclic and cyclic prochiral dicarboxylic acid diesters were also found to be good substrates for lipase-catalysed hydrolysis reactions. Examples are the hydrolyses of diesters **1.17**[68] and **1.19**[69] (Scheme 1.9).



Scheme 1.9. Enantiotopos-differentiating lipase-catalysed hydrolysis of dicarboxylic diesters

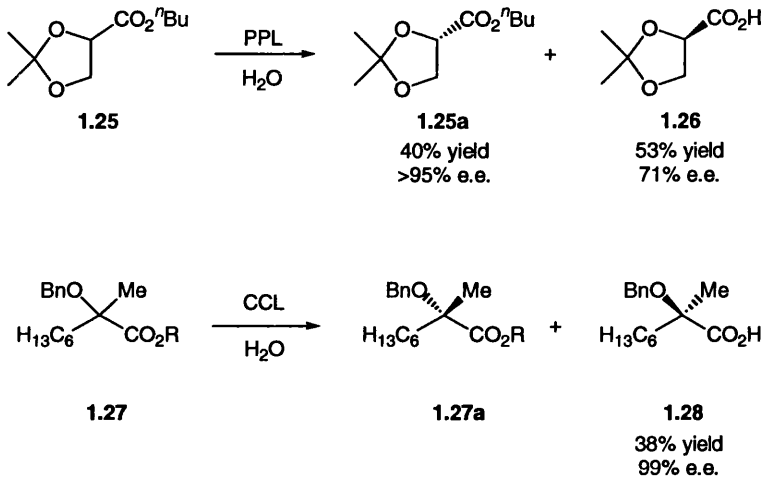
A second and no less important class of substrates for lipase-catalysed reactions is the racemates. The usefulness of lipases for the resolution of carboxylic acid esters and lactones by hydrolysis has been demonstrated.[2] Two examples are the lipase-catalysed hydrolysis reactions of racemic carboxylic acid **1.21**[70] and lactone **1.23**,[71] illustrated in Scheme 1.10.



Scheme 1.10. Resolution of carboxylic acid esters and lactones by lipase-catalysed hydrolysis

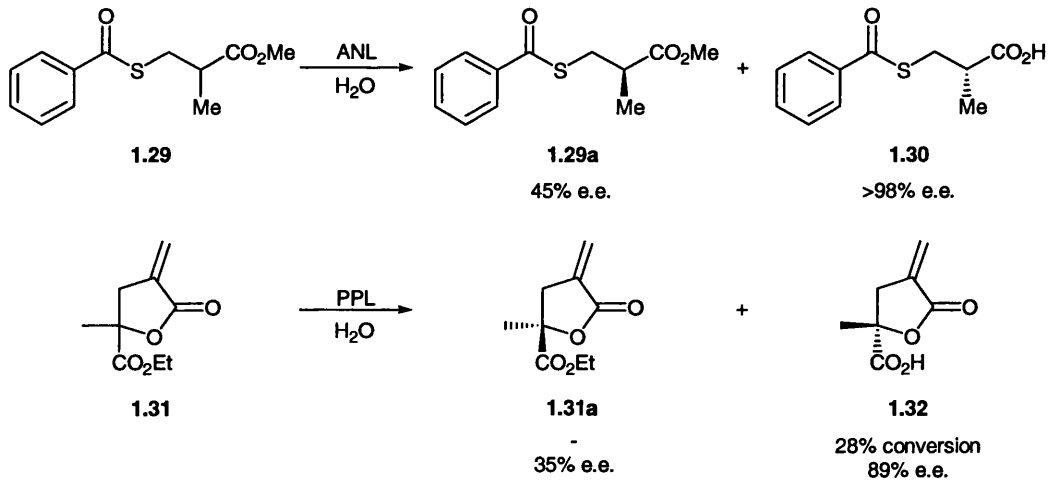
The broad substrate range, in the hydrolysis of carboxylic acid esters, is covered mainly by lipases from *Candida cylindracea* (*rugosa*), pig pancreas lipase and several *Pseudomonas* sp. lipases. Carboxylic acid esters having the alkoxycarbonyl group attached to a secondary, tertiary or even quaternary carbon atom are also

substrates. Thus, in contrast to uncatalysed ester hydrolysis, steric hindrance, at least for the known examples **1.25**[72] and **1.27**,[73] represents no problem (Scheme 1.11).



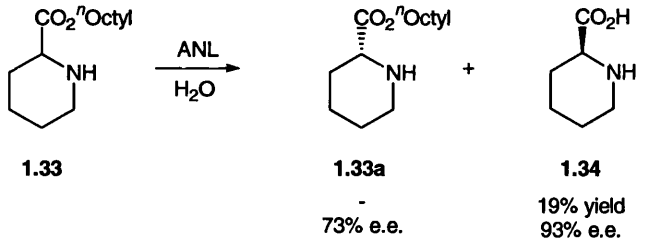
Scheme 1.11. Resolution of hindered racemic carboxylic esters

Functional group selectivity or chemoselectivity is also observed between an ester group and a thioester group or an ester and a lactone moiety as exemplified in Scheme 1.12 for compound **1.29**[74] and **1.31**.[75]



Scheme 1.12. Group selectivity in the hydrolysis of racemic carboxylic acid esters

α-Amino ester **1.33**, in Scheme 1.13, with unprotected amino group is hydrolysed to amino acid **1.34** with high enantioselectivity.[76]

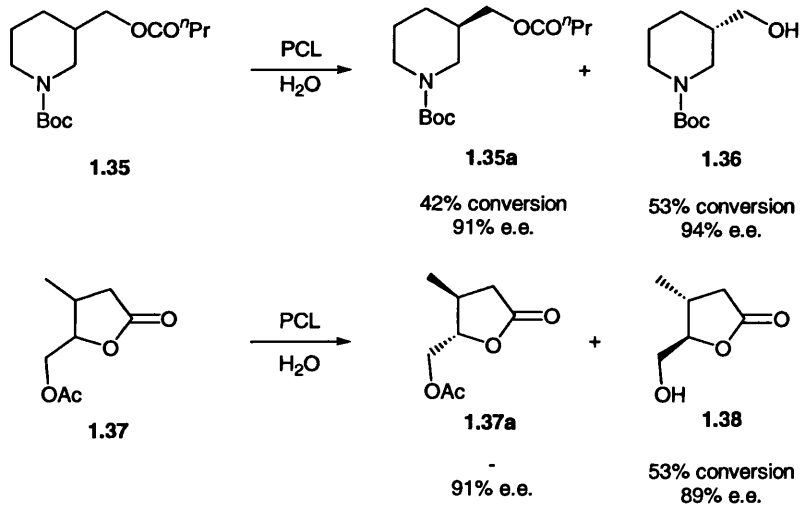


1.33 **1.33a** **1.34**

\- 73% e.e. 19% yield 93% e.e.

Scheme 1.13. Hydrolysis of unprotected amino acid **1.33**.

Lipase-catalysed resolution of acylated racemic primary alcohols by hydrolysis cover a broad range of substrates,[2] including amino alcohol **1.35**[77] and acylated γ-hydroxymethyl γ-lactone **1.37**[78] (Scheme 1.14).



1.35 **1.35a** **1.36**

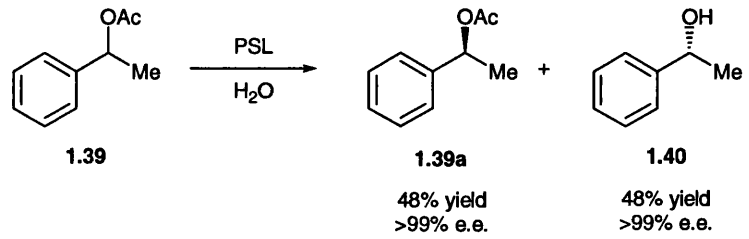
42% conversion 91% e.e. 53% conversion 94% e.e.

1.37 **1.37a** **1.38**

\- 91% e.e. 53% conversion 89% e.e.

Scheme 1.14. Lipase-catalysed hydrolysis of acylated primary racemic alcohols

Because of the experimental simplicity, lipase-catalysed resolution of racemic acylated secondary alcohols by hydrolysis reaction is today one of the best methods for the synthesis of enantiomerically pure secondary alcohols. There is almost no restriction in regard to the substrate structure. Highly enantiomer-selective hydrolysis of esters of a wide structural range of secondary alcohols by the different lipases is possible. An example[79] is illustrated in Scheme 1.15.

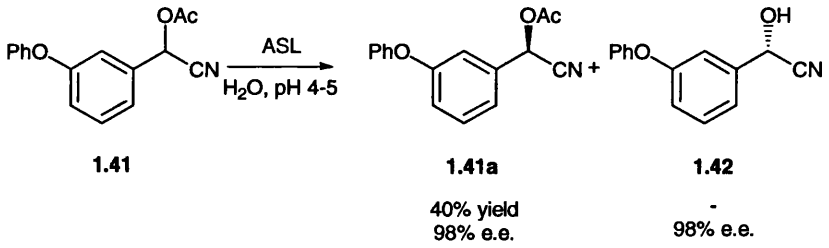


1.39 **1.39a** **1.40**

48% yield >99% e.e. 48% yield >99% e.e.

Scheme 1.15. Lipase-catalysed hydrolysis of acylated secondary racemic alcohols

A series of cyanohydrin acetates have also been prepared by a lipase-catalysed reaction. Isolation of the cyanohydrin itself is usually not possible because of the alkaline pH. With *Alcaligens* sp. lipase, whose pH optimum is between 4 and 5, isolation of the cyanohydrin acetate **1.41** as well as the cyanohydrin **1.42** becomes possible (Scheme 1.16).[80]

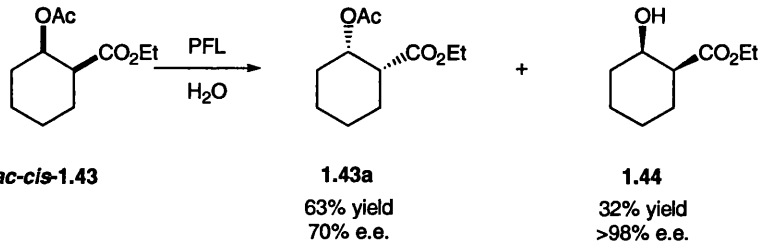


1.41 **1.41a** **1.42**

40% yield 98% e.e. - 98% e.e.

Scheme 1.16. Lipase-catalysed resolution of cyanohydrins

Several racemic secondary mono-, bi- and tricyclic acylated alcohols have also been resolved by a lipase-catalysed enantiomer differentiating hydrolysis. Usually these compounds bear a functional group in 2-position, such as compound **1.43** (Scheme 1.17).[81] The cycloalkanol can be *cis*- or *trans*-; hydrolysis is generally selective when both an acylated alcohol and a carboxylic acid ester are present in the molecule.

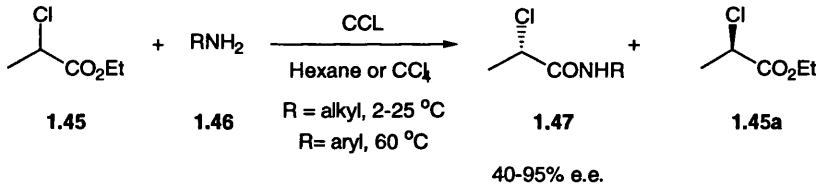


rac-cis-1.43 **1.43a** 63% yield 70% e.e. **1.44** 32% yield >98% e.e.

Scheme 1.17. Lipase-catalysed resolution of acylated cyclic racemic secondary alcohols

1.7.1.2 Hydrolysis and formation of N-O bonds

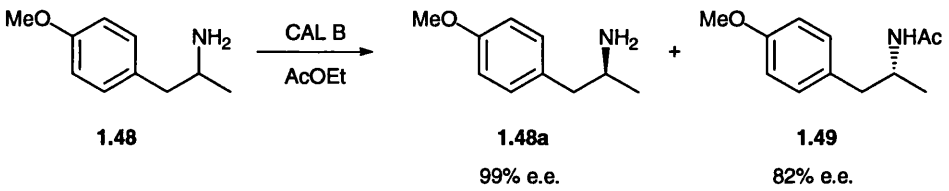
Lipases have been widely used for the preparation of enantiomerically enriched alcohols, esters and carboxylic acids through the corresponding asymmetric esterification and transesterification reactions (see previous paragraph). Recently, these enzymes have also been used in the preparation of enantiomerically pure amines and amides. Gotor and co-workers[82] have done most of the work, in this field. The utility of lipases for the preparation of enantiomerically pure amides was first demonstrated in the reaction of racemic chloropropionate with several aliphatic and aromatic amines (Scheme 1.18).[83]



1.45 **1.46** **1.47** 40-95% e.e. **1.45a**

Scheme 1.18. Lipase-catalysed preparation of enantiomerically pure amides from racemic chloropropionate

A series of pharmacologically interesting amines, such as amine **1.48** has been resolved by *Candida antarctica* lipase type B-catalysed enantioselective acylation (Scheme 1.19).[84]

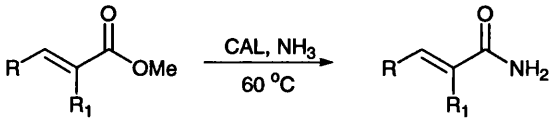


1.48 **1.48a** **1.49**

99% e.e. 82% e.e.

Scheme 1.19. Lipase-catalysed enantioselective acylation of amine **1.48**

N-unsubstituted acrylic amides **1.51** have also been prepared by a lipase-catalysed ammonolysis reaction (Scheme 1.20).[85]



R = H, Me, Ph, 2-Furyl
R_1 = H, Me

1.50

77-88% e.e.

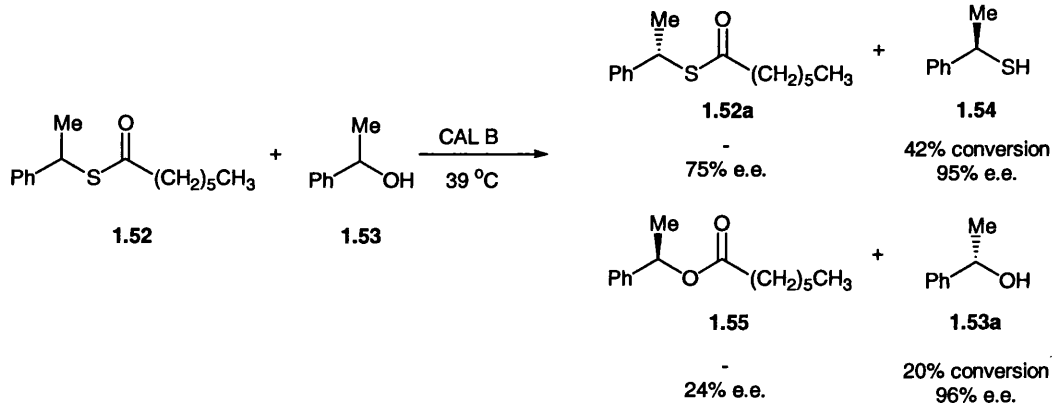
1.51

Scheme 1.20. Lipase-catalysed ammonolysis of esters

1.7.1.3 Hydrolysis and formation of S-O bonds

There are a few examples of lipase-catalysed kinetic resolution of thiols in the literature.

Phenylethanethiol **1.54** has been successfully resolved by a CAL B-catalysed transesterification reaction, as illustrated in Scheme 1.21.[8]



1.52 **1.53**

1.52a **1.54**

-
75% e.e.

42% conversion
95% e.e.

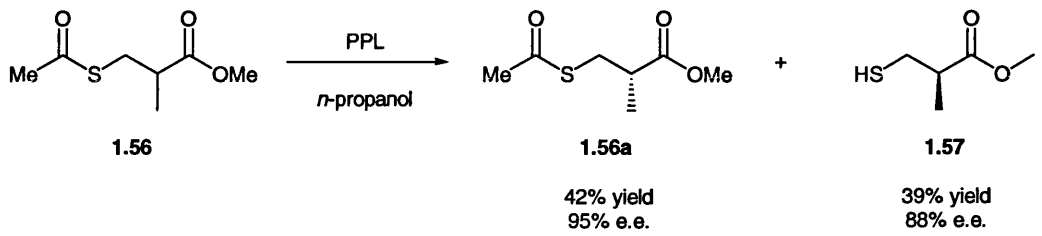
1.55 **1.53a**

-
24% e.e.

20% conversion
96% e.e.

Scheme 1.21. CAL B-catalysed resolution of phenylethanethiol **1.54**

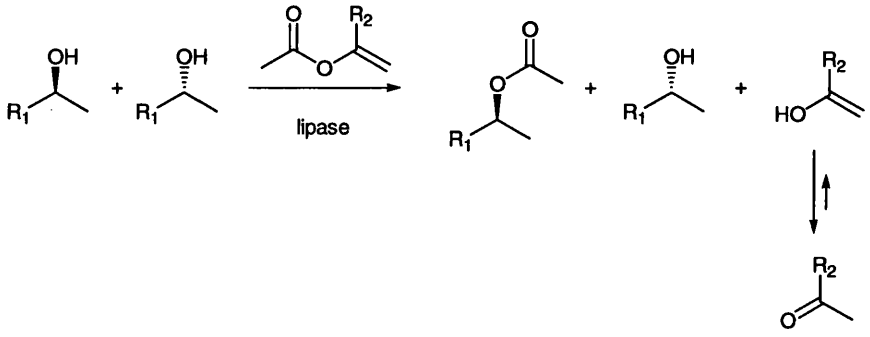
Mercapto ester **1.56** has been resolved by a lipase-catalysed transesterification, using *n*-propanol as organic solvent (Scheme 1.22).[86]



Scheme 1.22. Lipase-catalysed resolution of mercapto ester **1.56**

1.7.2 Selectivity of lipases towards organic substrates. Kinetic and Dynamic resolution

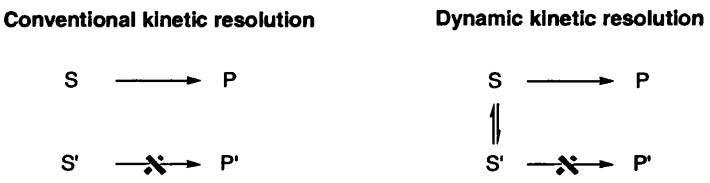
The success of a lipase-catalysed kinetic resolution is limited by the maximum chemical yield of 50% for each enantiomer (see above). However, as lipase-catalysed reactions are usually reversible, it must be kept in mind that the reverse reaction may lead to racemisation. Measures must be taken to drive the reaction in the desired direction. Thus, water liberated during esterification can be removed *in vacuo* or absorbed in molecular sieves. Alternatively, a large excess of the acyl donor can be introduced using, for example, ethyl acetate as both the acylating agent and the solvent. The method of choice to increase the reaction rate and to shift the equilibrium towards product synthesis is the use of activated esters such as vinyl acetate.[87-89] The alcohol freed from the transesterification rapidly tautomerizes to acetaldehyde or acetone, making the process irreversible (Scheme 1.23).



Scheme 1.23. Irreversible acylation with enol esters

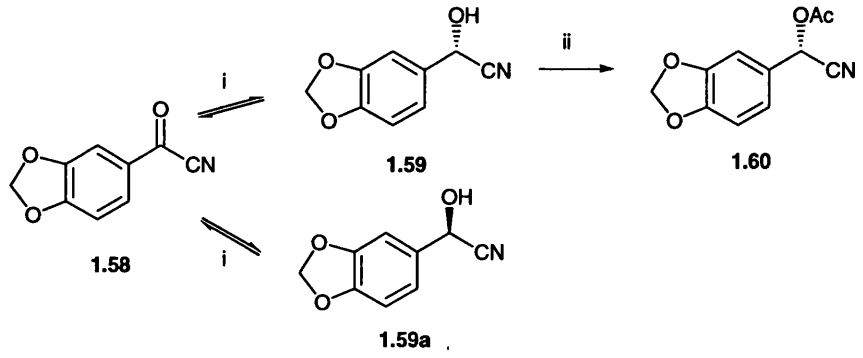
Which of the above-mentioned methods is used, the maximum conversion obtainable by a conventional kinetic resolution procedure remains 50%. This limitation can be overcome by a process called dynamic kinetic resolution (DKR).

The key idea of this principle is to racemise the slow reacting enantiomer continuously reproducing the faster one. In an ideal case, at the end of conversion one enantiomer is formed in 100% yield with 100% enantiomeric excess. This is illustrated in Scheme 1.24, where a conventional kinetic resolution and a dynamic kinetic resolution reaction are compared. In both cases enantiomer S reacts to form product P more quickly than enantiomer S'. In the conventional kinetic resolution enantiomer S' is simply left behind as unreacted starting material; in the dynamic kinetic resolution S and S' are in equilibrium and all the starting material can ideally be converted into product P. The reaction conditions must be chosen so that whilst the starting material enantiomers (S/S') undergo rapid equilibration (racemisation), the product P must be inert to racemisation.



Scheme 1.24. Conventional kinetic resolution and dynamic kinetic resolution

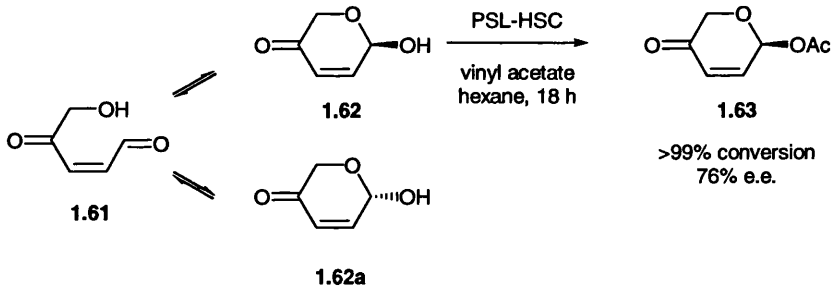
Lipases are very often used to achieve dynamic kinetic resolution.[90] Cyanohydrins, for example, are suitable substrates for dynamic kinetic resolution, because they are easily racemised with base. Several cyanohydrins, such as cyanohydrin **1.59**, formed by transhydrocyanation with acetone cyanohydrin, catalysed by the hydroxide form of an anion exchange resin, have been obtained in their enantiomerically enriched form by this method.[91,92] The reversible nature of cyanohydrin formation allows racemisation to proceed during the course of the lipase-catalysed acetylation, to afford acetylated cyanohydrin **1.60** in 81% yield and 91% enantiomeric excess (Scheme 1.25).



i) acetone cyanohydrin, IRA-904 resin (HO^- form)
ii) *Pseudomonas cepacia* lipase, isopropenyl acetate, 40 °C

Scheme 1.25. Lipase-catalysed dynamic kinetic resolution of cyanohydrins

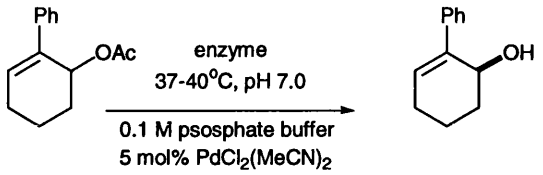
Successful dynamic kinetic resolution reactions have been performed using cyclic hemiacetals as substrates. The lipase-catalysed acetylation of 6-hydroxypyranone **1.61** has been achieved with reasonable enantioselectivity with essentially complete conversion (Scheme 1.26). The rate of reaction was found to greatly increase when the enzyme, lipase from *Pseudomonas* sp. was immobilized on Hyflo Super Cell (HSC).[93,94]



Scheme 1.26. Dynamic resolution of cyclic hemiacetals

However, most substrates for lipase-catalysed kinetic resolution reactions do not undergo spontaneous racemisation under conditions that are suitable for enzyme activity. To solve this problem, mild transition metal-catalysed methods for *in situ* racemisation has been designed. Racemisation must occur without affecting negatively the enzyme. At the same time, the enzyme must not inhibit the racemisation method. The first example was reported by Allen and Williams.[95] In this case, a palladium (II) catalyst was employed to racemise the allylic acetate substrate

1.64. The same catalyst did not effect the enantioselectivity of the lipase-catalysed reaction (Scheme 1.27).

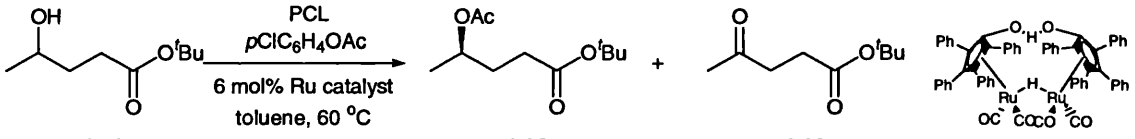


1.64

PFL: **(+)-1.65**, 96% e.e., 96% conv.
Acetylcholine esterase: **(-)-1.65** , 85% e.e., 68% conv.

Scheme 1.27. Dynamic kinetic resolution using transition metal/enzyme combinations

Bäckvall and co-workers have achieved successful results for a wide range of substrates, using different ruthenium catalysts. Their procedure works well for secondary alcohols containing aryl and alkyl groups,[96] diols, [97] and α-hydroxy esters.[98] An example of dynamic kinetic resolution of γ-hydroxy acid derivative **1.66**[99] is illustrated in Scheme 1.28.



1.66

1.67

70% yield
94% e.e.

1.68

3% yield
-

Ru catalyst

Scheme 1.28. Example of dynamic kinetic resolution of secondary alcohols

The challenge in the field of dynamic kinetic resolution is, of course, to discover racemisation methods that are compatible with the lipase-catalysed reaction.

1.8. References

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2. Evans' Auxiliaries. Features and General Applications

2.1 Introduction

Compounds that occur in Nature are enantiomerically pure because living organisms tend to produce only a single enantiomer of a given molecule. The asymmetry of these molecules arises from the inherent chirality of the enzymes that are responsible for their production.

Reception sites in biological systems, which are also enantiomerically enriched, have the ability to differentiate between two enantiomers of a specified molecule. If a pharmaceutical, or any biologically active compound, is chiral then the enantiomers are likely to interact differently with the natural biomolecule. The enantiomers will probably possess different levels of biological activity and could also exhibit quite different types of activity. In effect, the two enantiomers should be viewed as two distinct compounds. It follows that using racemate of a particular biologically active compound is equivalent to using a mixture of two different compounds.

Usually the reactivity of two enantiomers is different. This being the case, it is clearly undesiderable to use a racemic biologically active compound. Only one of the enantiomers possesses the desired beneficial activity, but both enantiomers carry the risk of unwanted activity (side effects). In the previous chapter we have described the utility of lipase-catalysed reactions in organic synthesis to obtain enantiomerically enriched alcohols, carboxylic acids, lactones and amines by kinetic resolution of racemic mixtures. Other enzymes than lipases are widely used to obtain a wide range of enantiomerically enriched compounds.[1,2]

However, the technique of resolving enantiomers has been used for well over a century before the introduction of enzymatic catalysis. In 1848 Louis Pasteur mechanically separated crystals of each optical isomer of sodium ammonium tartrate. Obviously this method is not suitable for a general resolution technique; nevertheless, selective crystallisation of one enantiomer is still used today as a method of resolution. Other methods of resolution involve the formation of a covalent bond between a racemate substrate and an enantiomerically pure molecule. The

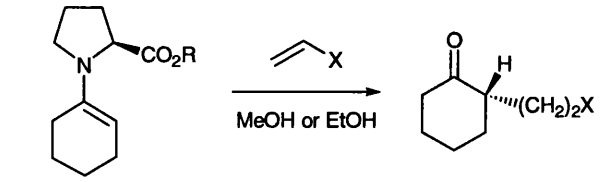
resulting pair of diastereoisomers, in many instances, can be separated by chromatographic techniques. Advances have recently been made in resolution techniques by chromatography of racemates on chiral stationary phases.[3]

Each of the methods of resolution described above suffer from the major disadvantage that half of the mixture is the wrong enantiomer. A quantitative separation in the resolution step at best allows for the recovery of only half of the synthesized material. Moreover, thus far, we have considered the separation of only one pair of enantiomers. As additional centres of asymmetry are added to the molecule, the number of stereoisomers increases exponentially by a factor 2^n. Therefore the separation of one particular stereoisomer from the mixture by resolution techniques is impractical, if not impossible, to achieve.

The solution to the aforementioned problems is asymmetric synthesis. Strategically, this can be accomplished by two basic approaches. First, the synthesis of a target molecule can be designed so as to incorporate a chiral fragment whose absolute stereochemistry is already established. In this respect Nature's "chiral pool" can be used to one's advantage. Second, the asymmetry in the target molecule can be induced by means of an external chiral auxiliary reagent that, under ideal conditions, is recoverable and recyclable. Again, Nature furnishes the source of chirality inherent in these auxiliaries.

Amino acids fulfil the criteria for both approaches to asymmetric synthesis.[4,5] Of all the classes of compounds of the "chiral pool", the amino acids as a family are the most versatile. Nearly all of the 19 common α-amino acids are available (many commercially) in two enantiomeric forms. Therefore synthetic routes can be planned to produce either enantiomer of a specified molecule. L-amino acids, which are commonly found in Nature, are the more abundant and hence less expensive of the two forms.

In 1969 Yamada and co-workers[6] reported the first stereoselective transformation of an amino acid derived enantiomerically pure enamine. In contrast to the modest chemical yields obtained, considerable selectivities were observed for the reaction of proline derivative **2.1** with α,β-unsaturated nitriles and esters (Scheme 2.1). The ketones, obtained after hydrolysis, were isolated with 43-59% enantiomeric excess.

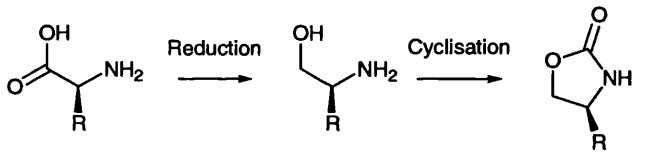


2.1
R = Me, Et, ^{t}Bu

2.2
X = CN, CO_2Me
43-59% e.e.

Scheme 2.1. Asymmetric synthesis of ketones from amino-acid derived enantiomerically pure enamine **2.1**

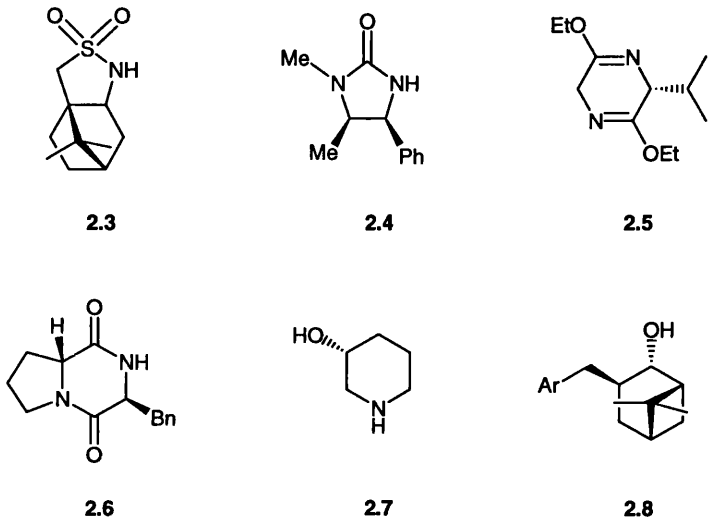
To perform useful chemical transformations using amino acids, it its often necessary to protect either or both reactive functionalities of the molecule, the amine and the carboxylic acid groups. In 1981 Evans and co-workers found a way to use intramolecularly protected α-amino acids as chiral auxiliary reagents.[7,8] An amino acid, usually valine, *tert*-leucine, phenylalanine or phenylglycine is reduced to the corresponding amino alcohol, which is then cyclised to the enantiomerically pure oxazolidinone (Scheme 2.2). The resultant 2-oxazolidinone is a stable and versatile chiral auxiliary reagent.



α-amino acid β-amino alcohol 2-oxazolidinone

Scheme 2.2. Preparation of Evans' chiral auxiliaries

The introduction of enantiomerically pure 2-oxazolidinones (Evans" chiral auxiliaries) can be considered a milestone in the history of asymmetric synthesis using covalently bound auxiliaries. Following the introduction of Evans" auxiliaries, other chiral auxiliaries such as Oppolzer's camphor derivatives (e.g. compound **2.3**)[9-11] carbohydrates,[12-14] ureas such as compound **2.4**,[15] the Schöllkopf chiral auxiliary **2.5**,[16-18] 2,5-diketopiperazine **2.6**,[19] (*R*)-piperidin-3-ol **2.7**,[20] chiral auxiliaries derived from (-)-β-pinene like compound **2.8**[21] (Scheme 2.3), and many others[22-25] have been designed and employed to achieve asymmetric syntheses.



2.3 2.4 2.5

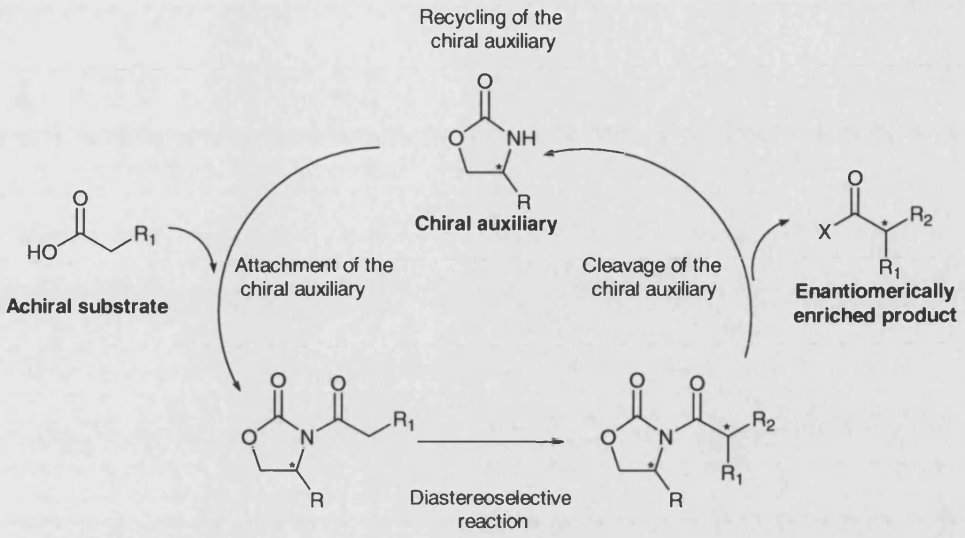
2.6 2.7 2.8

Scheme 2.3. Chiral auxiliaries used in synthesis

2.2 Chiral Evans' auxiliaries

2.2.1 Chiral auxiliary approach to asymmetric synthesis

Practical enantioselective transformations using chiral auxiliaries must be either catalytic, or the chiral auxiliary must be efficiently recycled. A general reaction cycle in a chiral auxiliary-based asymmetric synthesis is illustrated in Scheme 2.4. The achiral substrate is covalently bond to the chiral auxiliary. The acylated auxiliary then undergoes a diastereoselective reaction, which introduces a chiral centre in the achiral substrate. Finally, cleavage of the auxiliary affords enantiomerically enriched product.

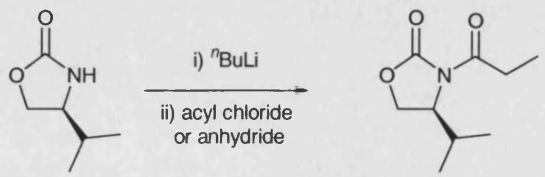


Scheme 2.4. Chiral auxiliary approach to asymmetric synthesis

An ideal chiral auxiliary has to fulfil several criteria in this overall reaction cycle. It should be inexpensive and both enantiomers should be readily available. Attachment and cleavage should proceed in high yield by simple methods. And, most importantly, it should provide a high degree of diastereoselection, being stable under the conditions of the diastereoselective reaction. If a chiral auxiliary fulfils all these criteria, at the end reaction cycle will afford enantiomerically enriched product and recyclable chiral auxiliary.

2.2.2 Evans' auxiliaries' stability: attachment and cleavage

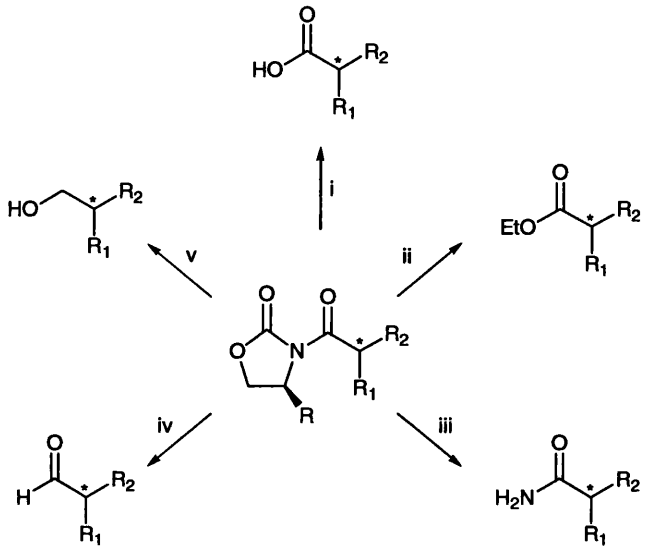
Despite the number of chiral auxiliaries today available, the strength of Evans' auxiliaries remains their close similarity to an ideal chiral auxiliary. In fact, as required, chiral 2-oxazolidinones are quite stable molecules. The strong base conditions usually required for the attachment of the achiral substrate to the auxiliary [7,26,27] don't affect the auxiliary (Scheme 2.5).



2.9 **2.10**

Scheme 2.5. Attachment of the auxiliary

In addition, cleavage of the auxiliary can be performed under mild reaction conditions affording the intact 2-oxazolidinone and a range of different classes of compounds, as identified in Scheme 2.6.[28-30]



i) LiOH, H_2O_2 ii) NaOEt iii) NH_3 iv) $LiBH_4$, H_2O v) $LiAlH_4$

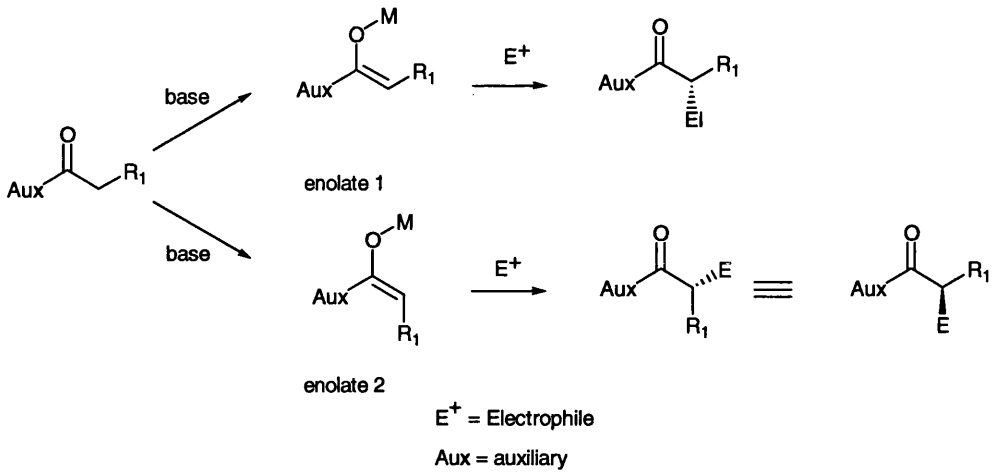
Scheme 2.6. Cleavage of the auxiliary

2.2.3 Evans' auxiliaries' main applications

Evans' auxiliaries also provide good diastereocontrol in different types of reaction widely used in asymmetric synthesis for the construction of C-C bonds.[4,28,31-33]

2.2.3.1 Stereoselective alkylation of chiral enolates

Substitution at the α position of chiral enolates is a large and important area of asymmetric synthesis. Several conditions are required for the alkylation of an enolate with high and predictable stereochemical control. First, the enolate has to be formed in only one of the two possible geometries. Considering the result of a non-selective enolate formation, illustrated in Scheme 2.7, reveals the importance of this. Even if the chiral auxiliary directs completely to one face of the enolate, the diastereoselectivity of the reaction will depend upon the preferred geometry in the enolate formation.

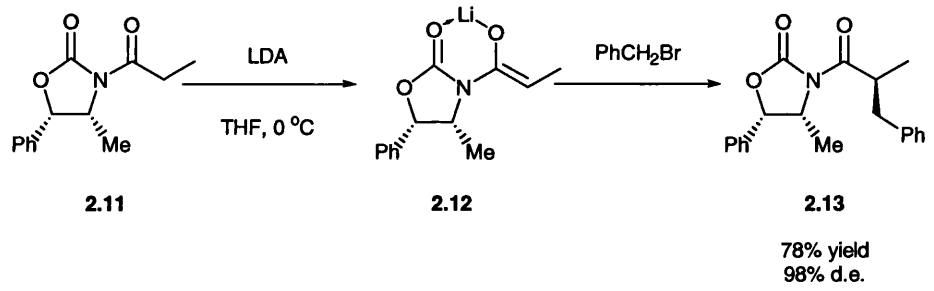


Scheme 2.7. Non-selective enolate formation in a chiral auxiliary based reaction

Thus, given that in Scheme 2.7 the chiral auxiliary directs reaction completely to the "upper" face of the enolate, two diastereomeric products are likely to be formed. If the selectivity is low, the asymmetric synthesis is unsuccessful.

Factors that can influence the stereoselectivity of enolate formation include choice of base, solvent, temperature, counter-ion and most importantly, the structure of the chiral auxiliary. Ideally, a given chiral auxiliary should also produce the same enolate geometry, exclusively enolate 1 or 2 in Scheme 2.7, irrespective of the nature of R_1.

A representative example of the high level of diastereoselectivity induced by Evans' auxiliaries is reported in Scheme 2.8. Treatment of acylated oxazolidinone **2.11** with base, usually LDA, produces chelated enolate **2.12** with 98% diastereomeric excess, to give almost exclusively product **2.13** as the major diastereomer.[34]



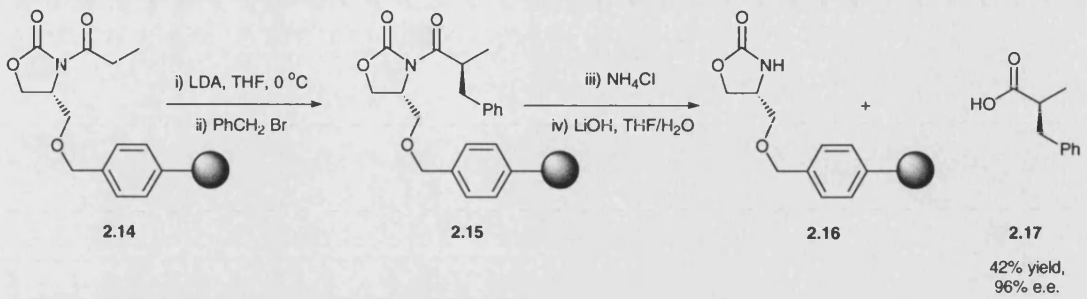
2.11 **2.12** **2.13**

78% yield
98% d.e.

Scheme 2.8. Oxazolidinones in stereoselective alkylations

Stereoselective alkylations have also been performed with an Evans-type supported oxazolidinone attached to a Merrifield polystyrene resin, for use in solid phase

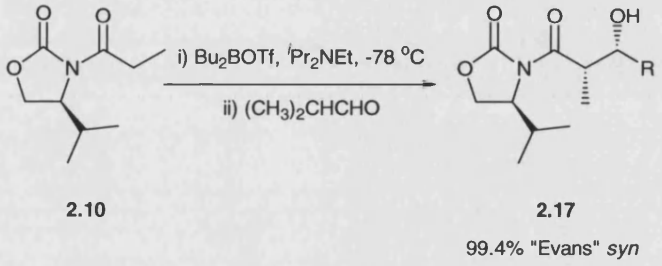
synthesis (Scheme 2.9).[35] After hydrolysis, the polymer bound auxiliary can be recovered by simple filtration.



Scheme 2.9. Polymer supported Evans' oxazolidinones in stereoselective alkylation reactions

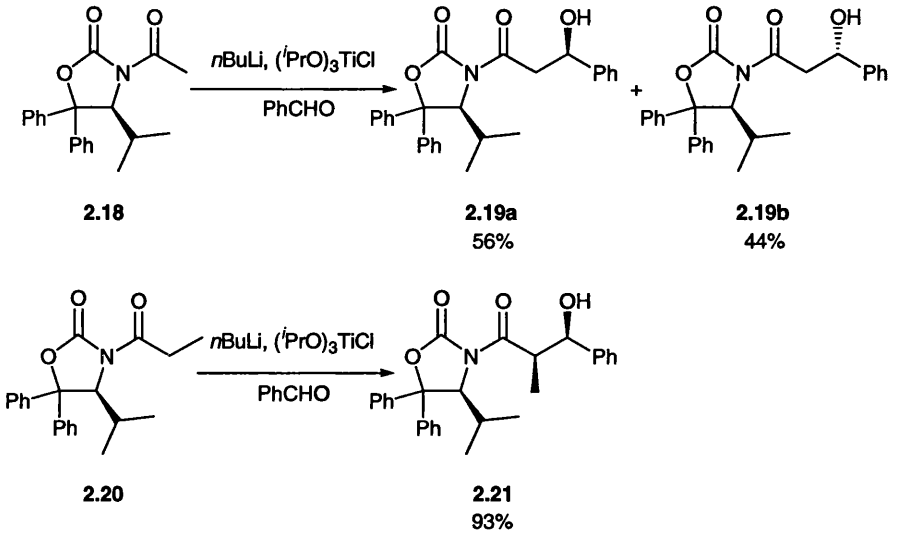
2.2.3.2 Stereoselective aldol reactions

The development of chiral enolates that participate in stereoregulated aldol condensations has been a challenging undertaking. Oxazolidinones have been able to fulfill most of the requirements (see Chapter 5). The utility of *Z*-enolates derived from *N*-acyl oxazolidinones has been shown through the aldol condensation reaction with aldehydes to give enantiomerically enriched α-substituted-β-hydroxy *N*-acyl-2-oxazolidinones in high yields. An example[7,26] is illustrated in Scheme 2.10.



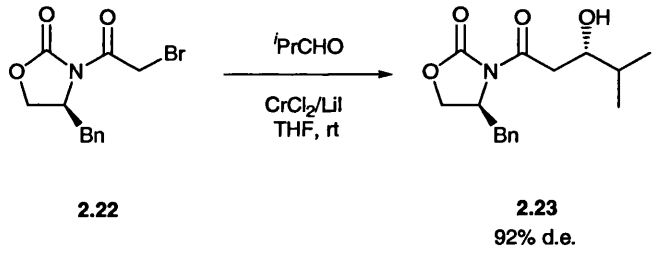
Scheme 2.10. Oxazolidinones in stereoselective aldol reactions

One of the problem of using acylated oxazolidinones in aldol reactions is that acetate enolates usually react relatively unselectively, when compared to the corresponding propionate enolates. This is illustrated in Scheme 2.11.[32]



Scheme 2.11. Comparison between diastereoselectivity of aldol reactions with acetate enolate and propionate enolate

To overcome this problem, α-bromoacetyl Evans' auxiliary **2.22** has been used in a chromium-Reformatsky reaction, affording product **2.23** with good diastereoselectivity (Scheme 2.12).[36]



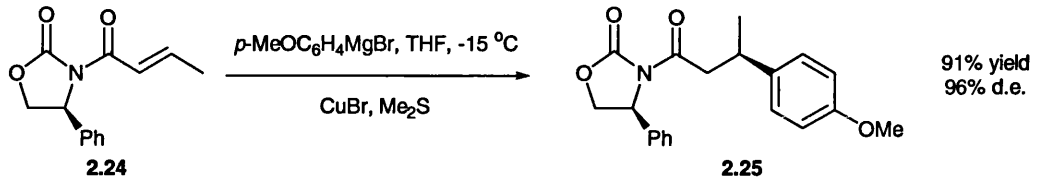
Scheme 2.12. Chromium-Reformatsky reaction using α-bromoacetyl Evans' auxiliary

2.2.3.3 Stereoselective conjugate additions and Diels-Alder reactions

Additions to C-C double bonds represent another important area in asymmetric synthesis, because several chiral centres can be formed in a single reaction. The most studied classes of reactions of this type are conjugate additions and Diels-Alder reactions.

The conjugate addition of organometallic reagents to crotonyl derivatives of a variety of chiral auxiliaries has been extensively studied. For example, conjugate additions of organocuprate to α,β-unsaturated *N*-acyl oxazolidinone **2.24** proceed with good

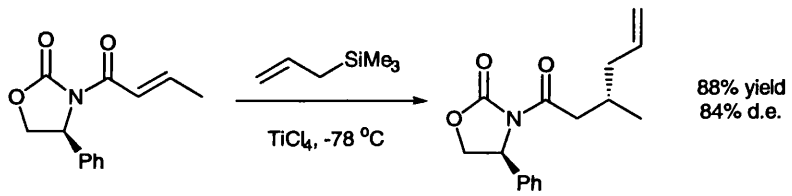
diastereoselectivity and allow access to β-branched carboxylic acids, as reported in Scheme 2.13.[37]



2.24 2.25

Scheme 2.13. Evans' auxiliaries in stereoselective conjugate additions

Allylated products such as compound **2.26** can also be prepared by the conjugate addition of allyltrimethylsilane, as shown in Scheme 2.14.[38]



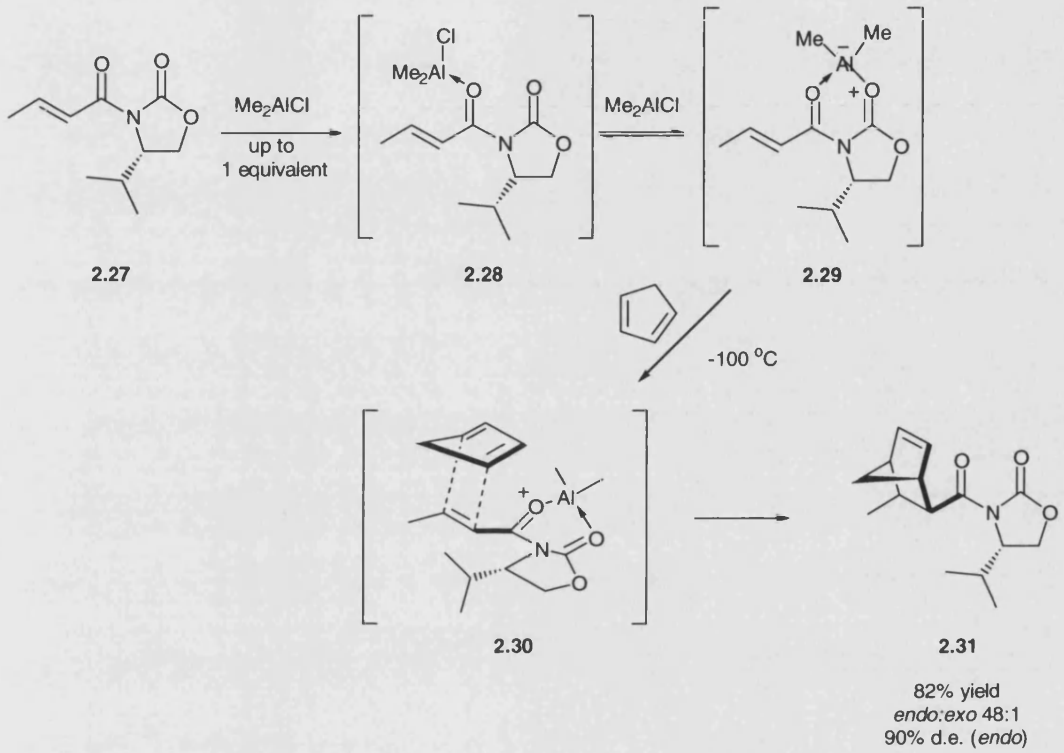
2.24 2.26

Scheme 2.14. Oxazolidinones in stereoselective allylations

The asymmetric Diels-Alder cycloaddition reaction can also be realized by use of chiral Evans' auxiliaries. The use of a dienophile attached to a chiral auxiliary has been the most intensively studied type of asymmetric Diels-Alder reaction. In the presence of dimethylaluminium chloride (DMAC), α,β-unsaturated *N*-acyl derivatives of enantiomerically pure oxazolidinones undergo *endo*-cycloaddition with high diastereoselectivity.

As previously discussed in relation to diastereoselective alkylations, chelation is important in understanding these reactions. The study of the reaction of oxazolidinone **2.27** with cyclopentadiene to give compound **2.31** (Scheme 2.15) as a function of the amount of added Lewis acid, shows that there is a dramatic increase in both the *endo:exo* ratio and in the diastereoselectivity of the *endo*-cycloaddition when one equivalent (or more) Lewis acid is present.[39] Furthermore, it also increases the reaction rate. This remarkable behaviour is interpreted in terms of initial formation of the complex followed by reversible ionisation promoted by the addition of more Lewis acid. This is illustrated in Scheme 2.15. The positively charged intermediate

2.29 formed on ionisation would be expected to be more conformationally constrained than the intermediate **2.28**, and would provide more stereocontrol in Diels-Alder reactions. A transition state resembling intermediate **2.30** seems to be responsible of the major adducts in these stereoselective reactions.[39-41]



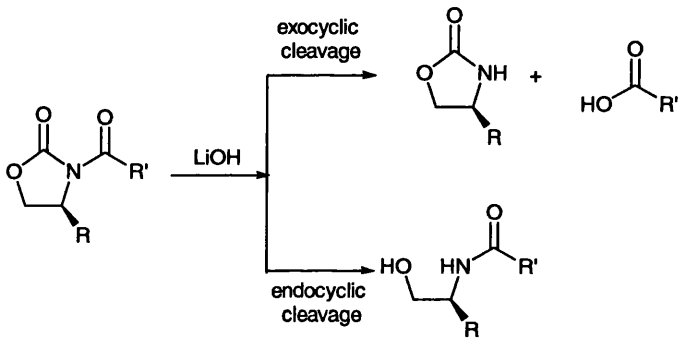
Scheme 2.15. Asymmetric Diels-Alder reactions with Evans' auxiliaries

Finally, some applications of chiral Evans' auxiliaries in stereoselective radical reactions are also reported.[42]

2.3 Towards the ideal chiral auxiliary. Chemically designed chiral Evans' auxiliaries

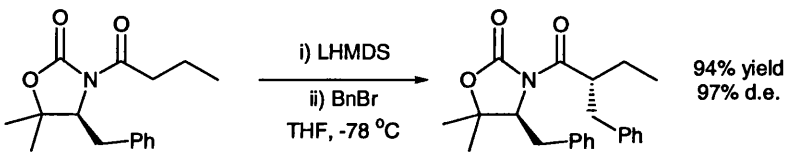
The ability to induce high stereocontrol in asymmetric synthesis is in practice the main reason why a specified chiral auxiliary is chosen to achieve asymmetric synthesis. That explains why since 1981 much work has been published about the use of novel Evans-type chiral auxiliaries, chemically designed and from other source than natural α-amino acids. Moreover, structural modifications often provide better stability under chiral auxiliary removal conditions.

One of the drawbacks of the Evans' methodology involves the removal of the auxiliary, which has to be done under mild and selective conditions, without racemisation of the stereogenic centres present in the system. The position of nucleophilic cleavage in *N*-acyl oxazolidinones is dependent upon steric and electronic requirements (Scheme 2.16).[43] The nucleophilic cleavage of unhindered systems is subject to electronic factors and exocyclic cleavage occurs to give the required products. If the *N*-acyl group is sterically demanding or α–branched, then the unwanted endocyclic hydrolysis can predominate. This problem can be overcome by using lithium hydroperoxide as a nucleophile, since it is apparently less susceptible to steric hindrance.[43]



Scheme 2.16. Cleavage of the auxiliary

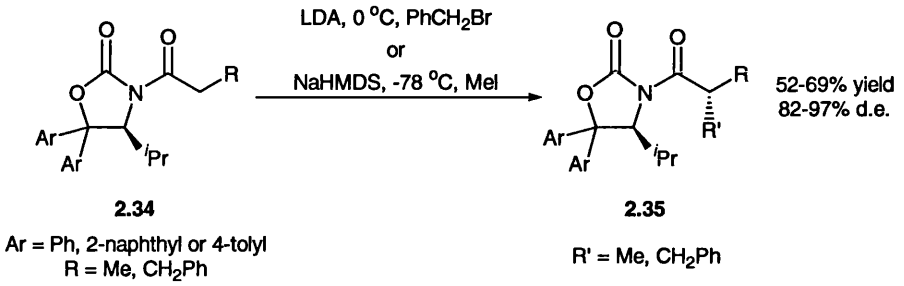
In 1995 Davies and co-workers[44] developed an elegant solution in the form of "SuperQuat " chiral auxiliaries, which are 4-substitued-5,5-dimethyl-2-oxazolidinones such as compound **2.32** (Scheme 2.17). These auxiliaries do not suffer from the undesired endocyclic cleavage and give very good diastereoselectivities in alkylation and conjugate addition reactions.[45,46] An example[47] is illustrated in Scheme 2.17.



2.32 **2.33**

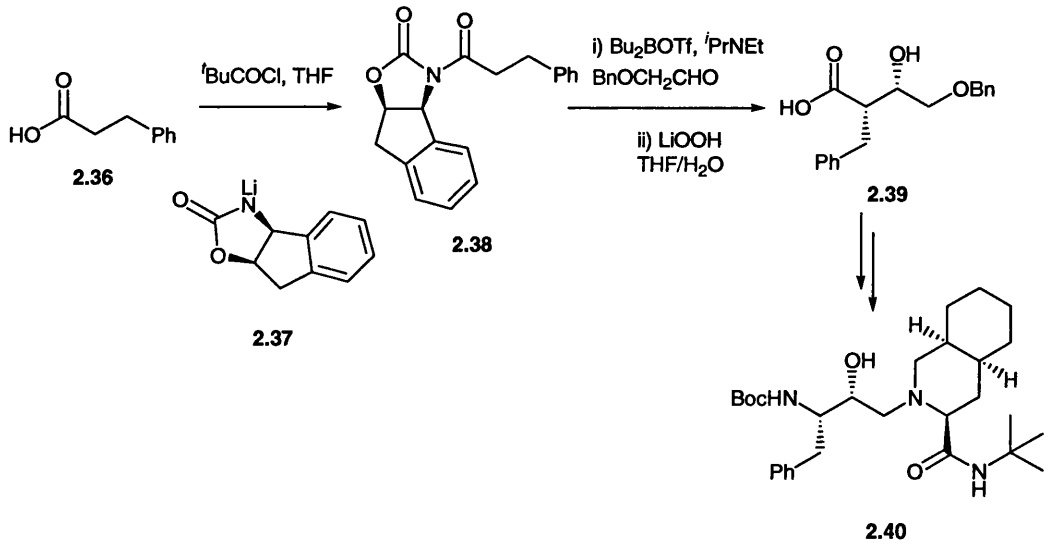
Scheme 2.17. Stereoselective alkylation with a "SuperQuat" auxiliary

Other *N*-acylated oxazolidinones, with different substituents than methyl groups in 5 position, such as oxazolidinones **2.34** (Scheme 2.18), have been studied and tested for their ability to promote diastereoselective reactions.[48]



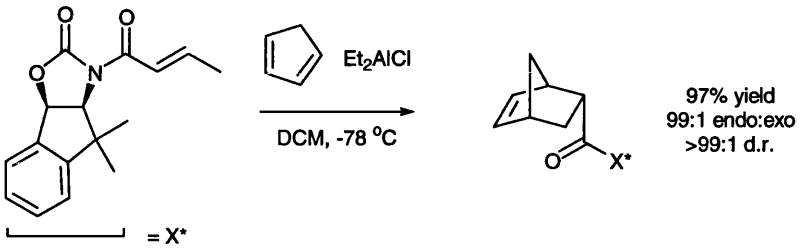
Scheme 2.18. Other "SuperQuat" auxiliaries

Another important class of Evans'-like chiral auxiliaries is that of amino indanol derivatives.[49,50] The amino indanol derived auxiliary **2.37**, prepared by treatment of amino indanol with disuccinimidyl carbonate in the presence of triethylamine, promotes aldol reactions with complete diastereofacial selectivity (>99% d.e.)[51] An example of application of this amino indanol derived auxiliary in the synthesis of the core unit of the HIV protease inhibitor Sequinavir® **2.40** is reported in Scheme 2.19.[52]



Scheme 2.19. Application of the *cis*-1-amino indan-2-ol derived auxiliary

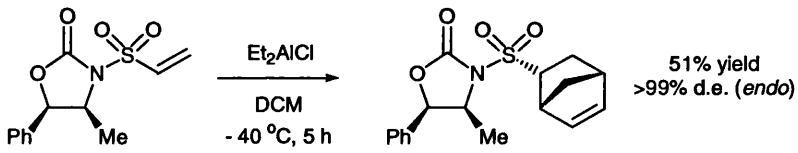
The aminoindanol-derived oxazolidinone **2.41** (Scheme 2.20) has a *gem*-dimethyl group on one face of the alkene and afforded >99:1 selectivity in Diels-Alder reactions with both cyclic and acyclic dienes, using Et_2AlCl as Lewis acid.[53]



2.41 **2.42**

Scheme 2.20. Aminoindanol-derived oxazolidinone**2. 41** in Diels-Alder reactions

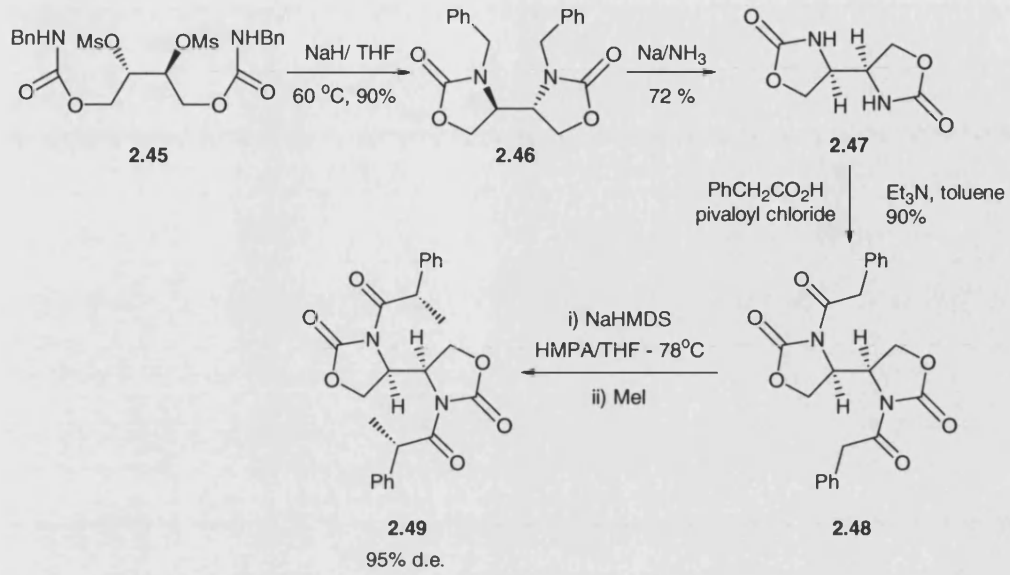
The enantiomerically pure oxazolidinone-derived vinylsulfonamide **2.43** (Scheme 2.21) has also been employed in asymmetric Diels-Alder reaction with cyclopentadiene, again using Et_2AlCl as Lewis acid, but in this case increasing the temperature to up to -40 °C.[54]



2.43 **2.44**

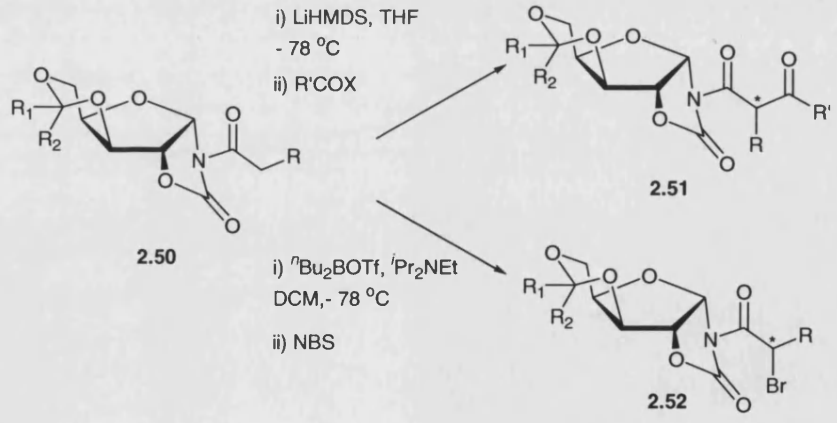
Scheme 2.21. Oxazolidinone-derived vinylsulfonamide **2.43** in Diels-Alder reactions

C_2-symmetric bis(oxazolidinone) **2.47**, the first water-soluble chiral auxiliary,[55] has been synthesized via intramolecular regioselective cyclisation of biscarbamate **2.45**. The sodium enolate from *N,N'*-di(phenylacetyl)bis(oxazolidinone) **2.48** reacts with methyl iodide with high facial selectivity (95:5) (Scheme 2.22).



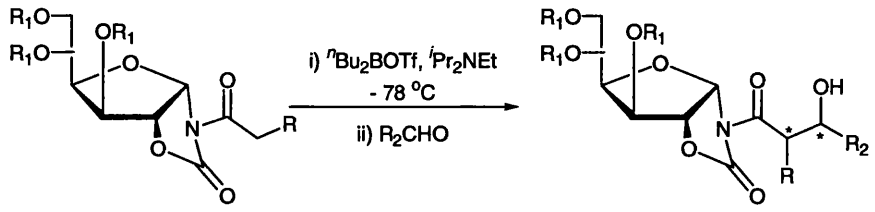
Scheme 2.22. Application of the water-soluble C_2-symmetric bis(oxazolidinone) **2.47**

Oxazolidinone-type chiral auxiliaries have also been synthesized from carbohydrates. Chiral *N*-acylated 2-oxazolidinones readily available from D-xylose have been demonstrated to undergo highly diastereoselective acylation reactions.[56] The glyco-2-oxazolidinones **2.50**, illustrated in Scheme 2.23, can also be used in diastereoselective halogenation reactions via their boron-enolate to get α-halogenated products.



Scheme 2.23. Applications of D-xylose derived 2-oxazolidinones

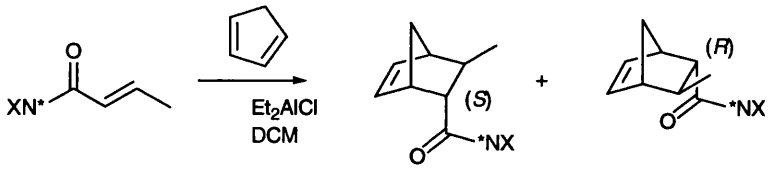
Other glyco-2-oxazolidinones based on D-glucose **2.53** have been used in dialkylboron-mediated aldol reactions (Scheme 2.24).[57]



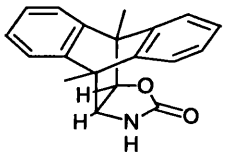
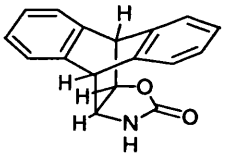
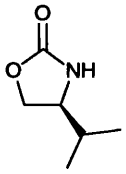
2.53 **2.54**

Scheme 2.24. D-glucose derived 2-oxazolidinones mediated aldol reactions

Enantiomerically pure [4+2]cycloadduct-based-2-oxazolidinones **2.57** and **2.58** (Scheme 2.25) were used as excellent chiral auxiliaries in the Evans' asymmetric strategy, providing very high diastereoselectivity in alkylations and Diels-Alder reactions, when compared with traditional Evans' auxiliaries.[58-59] An example[59] of cycloaddition reaction is reported in Scheme 2.25.



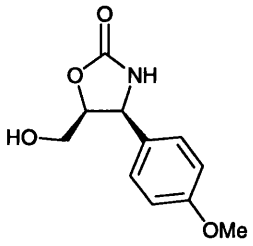
2.55 (*S*)-2.56 (*R*)-2.56

HXN*	T (°C)	Yield (%)	*exo:endo*	*endo* (*S*)-2.56:(*R*)-2.56
 2.57	- 78	97	1:99	1:327
 2.58	- 78	100	1:49	1:55
 2.59	- 100	82	1:48	1:32

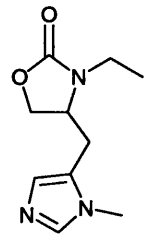
Scheme 2.25. [4+2]Cycloadduct-based-2-oxazolidinones as Excellent chiral auxiliaries

2.4 Other uses of enantiomerically pure 2-oxazolidinones

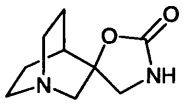
The above paragraphs described the use of chiral 2-oxazolidinones in different efficient and elegant asymmetric syntheses. In conclusion, it should be mentioned that in the last few years enantiomerically pure 2-oxazolidinones have also received wide attention for their high biological activity. Some significant examples of drugs, which incorporate the oxazolidinone ring in their active structures, are displayed in Scheme 2.26.



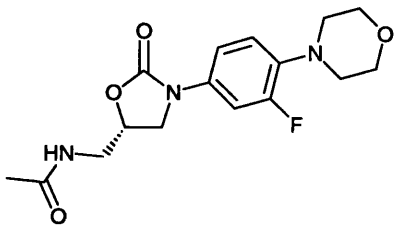
cytotaxone **2.60**



pilocarpine analogue **2.61**



AR-R17779 **2.62**



linezolid **2.63**

Scheme 2.26. Synthetic biologically active 2-oxazolidinones

An example is cytotaxone **2.60**, an alkaloid isolated from *Streptomyces* sp. This oxazolidinone is an immunomodular that inhibits intercellular communication between macrophages.[60] An oxazolidinone analogue of the muscarinic agonist pilocarpine (compound **2.61**) is equipotent to pilocarpine andused for the treatment of glaucoma. This means that the oxazolidinone analogue has the flexibility to adopt the necessary receptor-active configuration.[61]

Another series of oxazolidinones receiving a lot of interest are the spirooxazolidinones. AR-R17779 **2.62** has been found to be a strong antagonist of the rat a7 nicotinic receptor. Such antagonists have been used in the treatment of neurodegenerative diseases such as Alzheimer's disease.[62]

The oxazolidinones are also a new chemical class of synthetic antimicrobial agents with a unique mechanism of inhibiting bacterial protein synthesis. Their activity against multi-drug resistant gram-positive pathogenic bacteria has been used to develop a new class of potent antibiotics. Linezolid **2.63**[63,64] is shown in Scheme 2.26.

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3. Preparation of 2-Oxazolidinones by Enzymatic Desymmetrisation

3.1 Introduction

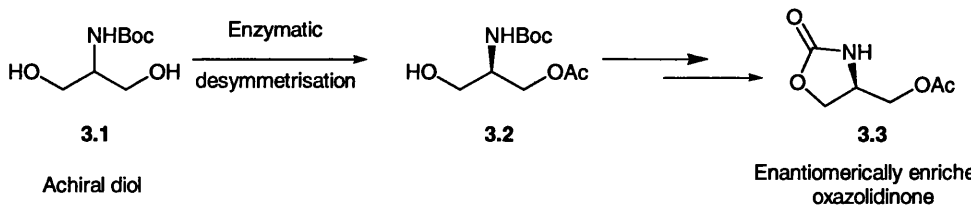
Desymmetrisation of *meso*-configured or prochiral substrates to obtain enantiomerically enriched products is a powerful synthetic tool. The advantage of desymmetrisation over conventional kinetic resolution reactions being the potential ability to achieve high enantiomeric excess even at 100% conversion.[1]

In general, the desymmetrisation of two enantiotopic functional groups can be achieved using a chiral reagent or catalyst.

The ready availability of *meso*-diols by the *cis*-dihydroxylation of suitable alkenes and their utility for the preparation of chiral building blocks make them popular substrates for enantioselective desymmetrisation studies.[2-5] Desymmetrisation of achiral diols using enzymes is a well known process for the formation of enantiomerically enriched mono-esters.[6-9]

This chapter focuses upon the enzymatic desymmetrisation strategy, which has been successful in the preparation of enantiomerically enriched oxazolidinones.

Our overall desymmetrisation strategy involved the use of *N*-Boc-protected serinol **3.1** to give a mono-acetate **3.2**, followed by appropriate chemical transformation to afford enantiomerically enriched oxazolidinones (e.g. compound **3.3**) (Scheme 3.1).



3.1 Achiral diol

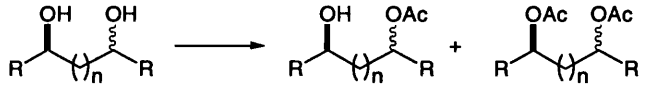
3.2

3.3 Enantiomerically enriched oxazolidinone

Scheme 3.1. General approach to preparation of oxazolidinones by enzymatic desymmetrisation

3.2 Background. Enzymatic desymmetrisation of diols

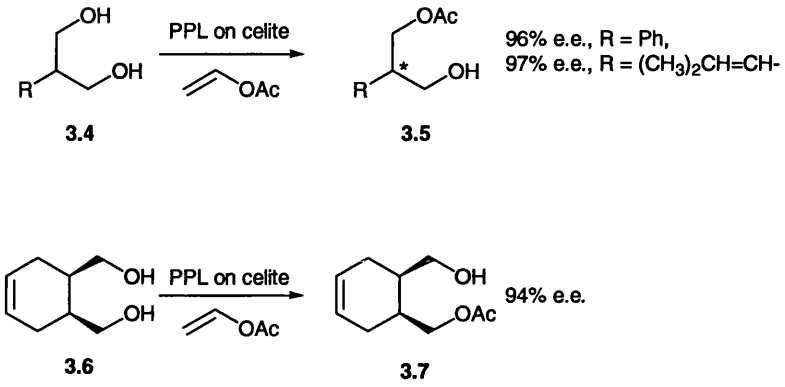
The mono-functionalisation of diols is an important challenge in organic synthesis.[1] Whilst sterically or electronically different hydroxyls may be selectively modified, chemical methods for the mono-functionalisation of similar hydroxyls groups are not so many. The problem is that both hydroxyls groups react at a similar, if not the same, rate and thus even with one equivalent of reagent, a statistical mixture of starting diol and mono-/bis functionalised products are formed (Scheme 3.2).



Scheme 3.2. Desymmetrisation of diols

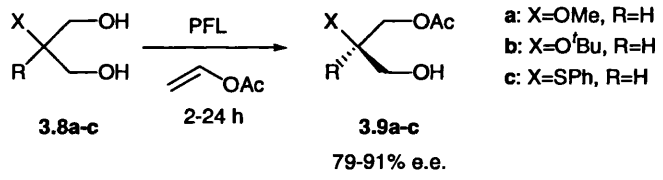
Enzyme-catalysed acetylation of diols in organic solvent has rapidly become one of the best methods to achieve desymmetrysation of *meso*-diols.
Porcine pancreas lipase (PPL) represents the cheapest commercially available lipase. Although it is a complex mixture of different hydrolases and is mainly used in the hydrolysis of esters, many successful applications of this crude preparation for the desymmetrisation of prochiral diols by selective mono-acetylation in organic solvent have been achieved. Other lipases of microbial origin, like those from *Pseudomonas sp.* and *Candida sp.* strains have shown synthetic utility in the acylation of diols in organic solvent.[10]

Lipase-catalysed acetylation is a widely applied method for the desymmetrisation of 2-substituted-1,3- diols. For example, Guanti and co-workers[11] reported the desymmetrisation of a series of 2-substitued-1,3-propanediols and of a *cis*-cyclohexene diol through monoacetylation with crude PPL supported on celite. Vinyl acetate acted as both the solvent and acylating agent (Scheme 3.3).



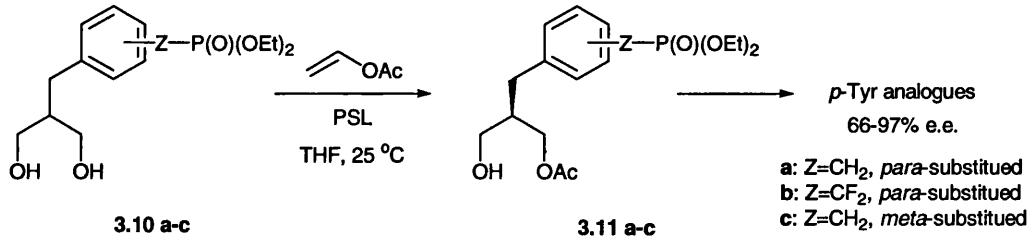
Scheme 3.3. Enzymatic desymmetrisation of propanediols **3.4** and *cis*-cyclohexene diol **3.6**

Meso-1,3-diols disubstituted with an alkoxymethyl or a thiophenyl group in the 2 position have been converted into the corresponding monoacetate through an enzyme-catalysed acylation with *Pseudomonas fluorescens* lipase (Scheme 3.4).[12]



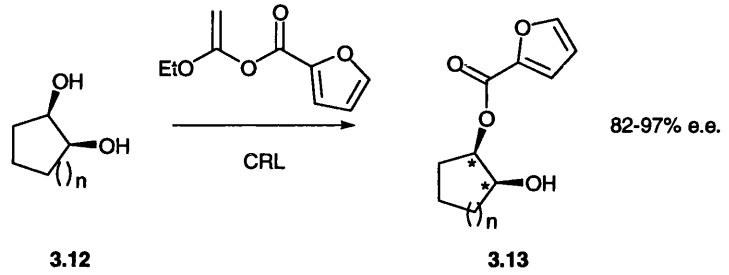
Scheme 3.4. Enzymatic desymmetrisation of 2-disubstituted-1,3-diols

Phosphorylated tyrosine analogues can be obtained by *Pseudomonas sp.* catalysed desymmetrisation of 2-benzyl-1,3-propanediols **3.10** possessing different functionalities at the *para*- or *meta*-position (Scheme 3.5).[13]



Scheme 3.5.Preparation of tyrosine analogues by desymmetrisation of 2-benzyl-1,3-propanediols **3.10**

Meso-cis-1,2-cycloalkanediols **3.12** were efficiently desymmetrised with 82-97% e.e. without racemisation by lipase from *Candida rugosa* (Scheme 3.6).[14]

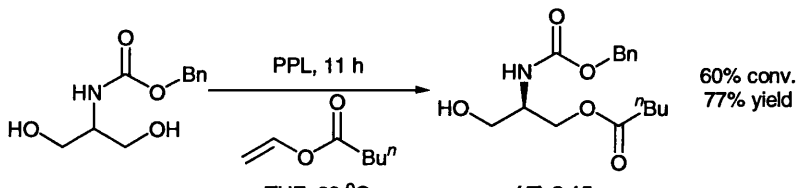


3.12 **3.13**

Scheme 3.6. Desymmetrisation of *meso-cis*-1,2-cycloalkanediols

Several other examples of application of lipases in the desymmetrisation of diols have been reported.[15-19]

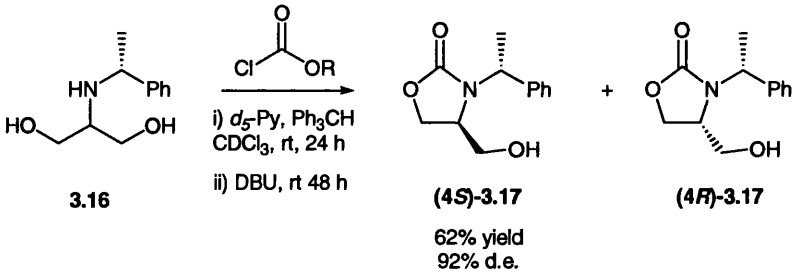
Serinol derivatives have only received limited attention in desymmetrisation reactions. A literature search reveals few examples in which serinol has been used as starting material in desymmetrisation-based syntheses.
Enantiomerically pure 3-*O*-acetyl-2-*N*-(benzyloxycarbonyl)serinol **3.15**, an important building block for the preparation of biologically active molecules such as phospholipids, phospholipase A2 inhibitors and sphingoglycolipids[20] can be prepared by PPL-catalysed desymmetrisation of 2-O-benzyl 2-*N*-(benzyloxycarbonyl)serinol **3.14** (Scheme 3.7).[21]



3.14 (*R*)-**3.15**

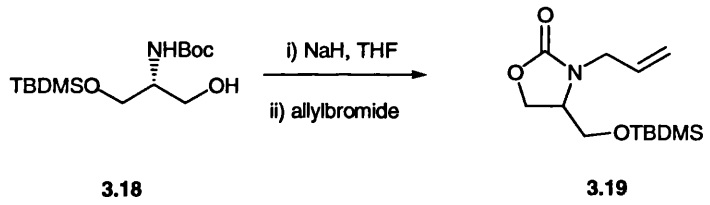
Scheme 3.7. Desymmetrisation of 3-*O*-acetyl-2-*N*-(benzyloxycarbonyl)serinol **3.14**

A derivative of serinol possessing an enantiomerically pure α-methyl benzyl nitrogen protecting group, compound **3.16**, has been used in a chemical/auxiliary based diastereoselective synthesis of oxazolidinones. Treatment with chloroformate afforded **(4*S*)-** and **(4*R*)-3.17** with up to 92% d.e. (62% yield) (Scheme 3.8).[22]



Scheme 3.8. Asymmetric synthesis of serinol derivatives

One potential problem in the preparation of desymmetrised serinol derivatives is that of racemisation by exchange reactions. In one example, racemisation during oxazolidinone formation of a mono-silyl ether of *N*-Boc-serinol has been reported and a 1,3-silyl shift was suggested in this case as a likely cause of racemisation (Scheme 3.9).[23]

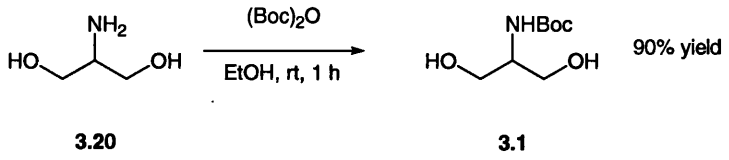


Scheme 3.9. Oxazolidinone formation of a mono-silyl ether of *N*-Boc-serinol

We decided to focus our attention upon the enzymatic desymmetrisation of serinol as potential precursor of enantiomerically enriched oxazolidinones.

3.3 Desymmetrisation of *N*-Boc-serinol

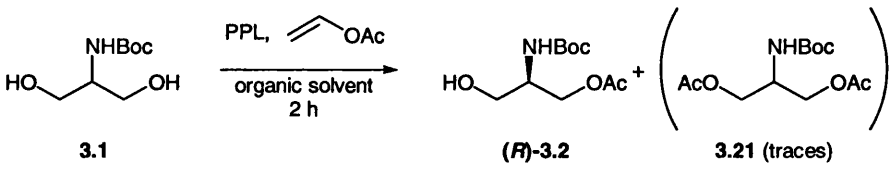
Desymmetrisation of serinol (1,3-dihydroxy-2-aminopropane) required previous protection of the amino group to avoid *N*-acylation.[24,25] Boc-protection of achiral serinol **3.20** was achieved in 90% yield by treatment of serinol with Boc anhydride (Scheme 3.10).[26]



3.20 **3.1**

Scheme 3.10. Boc-protection of achiral serinol **3.20**

The desymmetrisation of *N*-Boc-serinol was achieved by the selective mono-acetylation using PPL (porcine pancreas lipase) and vinyl acetate as the acylating agent, in organic solvent (Scheme 3.11). A small amount of di-acetylated material was also observed, providing a self-correcting process for the removal of the unwanted enantiomer of mono-acetylated product. [27]



3.1 **(*R*)-3.2** **3.21** (traces)

Scheme 3.11. Desymmetrisation of *N*-Boc-serinol by PPL

Only one enantiomer of the mono-acetylated product was detected by chiral HPLC. The effects of solvent and temperature variations on the conversion and selectivity of the acylation reaction were investigated.

We first examined the effect of solvent on the acylation of protected serinol **3.1** at 30 °C (Table 3.1).

Table 3.1. Influence of solvent on conversion, e.e. and mono/di-acetylated product ratio[a,b] for reaction illustrated in Scheme 3.11

Entry	Solvent	Conversion (%)	e.e. (%)	Mono/ di-acetylated[c]
1	THF/*n*-hexane 1:1	96	>99	87
2	$^{i}Pr_2O$	42	>99	>99
3	Vinyl acetate	>99	>99	>99
4	Cyclohexane	28	>99	>99
5	Toluene	29	>99	>99
6	Acetone	72	>99	98
7	Acetonitrile	28	>99	>99

[a] 140 $mg_{enzyme}/mmol_{substrate}$ in 5 mL solvent using 1 mmol of *N*-Boc-serinol and 3 mmol vinyl acetate.

[b] Conversion, enantiomeric excess and mono/di-acetylated ratio were determined by HPLC analysis.[28]

[c] Mono/di-acetylated ratio = = [(*R*)-**3.2**/**3.21**] x 100.

As can be seen (entries 1 and 3), striking differences in reactivity were found, vinyl acetate and a mixture of THF/*n*-hexane being the best reaction solvents. The increase in conversion was particularly evident when compared to solvents such as cyclohexane, toluene and acetonitrile (entries 4, 5, 7).

The reaction in vinyl acetate was examined at different reaction temperatures. In Table 3.2 the relative results after 2 h are displayed.

Table 3.2. Influence of temperature on conversion, e.e. and mono/di-acetylated product ratio[a, b] for reaction illustrated in Scheme 3.11

Entry	Temperature (°C)	Conversion (%)	e.e. (%)	Mono/ di-acetylated[c]
1	30	>99	>99	>99
2	40	>99	>99	85
3	50	>99	>99	84

[a] 140 $mg_{enzyme}/mmol_{substrate}$ in 5 mL vinyl acetate using 1 mmol of *N*-Boc-serinol.

[b] Conversion, enantiomeric excess and mono/di-acetylated ratio were determined by HPLC analysis.[28]

[c] Mono/di-acetylated ratio = = [(*R*)-**3.2**/**3.21**] x 100.

Increasing the temperature (entries 2 and 3) had a noticeable effect upon the di-acetylated product, which appears after only 2 h.

Although PPL is widely used for the resolution of prochiral diols,[29] we also examined other enzymes in our desymmetrisation strategy. Lipase from *Candida antarctica* type B (CAL B), lipase from *Candida cylindracea* (CCL), lipase from *Pseudomonas cepacia* (PCL) and from *Pseudomonas fluorescens* (PFL) immobilized on Sol-Gel-AK, lipase from *Aspergillus niger* (ANL) were tested for their activity and selectivity in the acylation reaction illustrated in Scheme 3.11. Again vinyl acetate was used as both the acylating agent and solvent at 30 °C (Table 3.3).

Table 3.3. Influence of catalyst on conversion, e.e. and mono/di-acetylated product ratio[a] of reaction illustrated in Scheme 3.11

Entry	Lipase	Conversion (%)	e.e. (%)	Mono/ di-acetylated[c]
1	PPL	>99	>99	>99
2	CAL B[b]	>99	1	<1
3	CCL	49	>99	94
4	PCL	96	>99	91
5	ANL	16	>99	>99
6	PFL	96	>99	92

[a] For reaction conditions see Table 3.1.

[b] Reaction with CAL B was performed in both vinyl acetate and THF/*n*-hexane solution.

[c] Mono/di-acetylated ratio = [(***R***)-**3.2**/**3.21**] x 100.

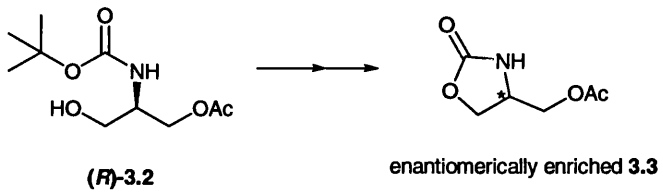
The conversion after 2 h was greater than 96% when PPL, CAL B, PCL and PFL (entries 1, 2, 4, 6) were used as catalysts. The best selectivity was observed with PPL.

No selectivity was observed with CAL B. The di-acetylated product only was formed in reactions in both vinyl acetate and THF/*n*-hexane media (Table 3.3, entry 2).

In summary, the maximum conversion (>99%, after 2 hours) and selectivity (>99% e.e. and >99% mono/diacetylated product) were obtained when the reaction was performed in vinyl acetate at 30 °C, using porcine pancreas lipase as catalyst. The isolated yield of product after chromatographic purification was 69%.

3.4 Synthesis of enantiomerically enriched oxazolidinones

The next step in our study was to establish the cyclisation of acetate **3.2** to obtain enantiomerically enriched oxazolidinone **3.3**, as illustrated in Scheme 3.12.

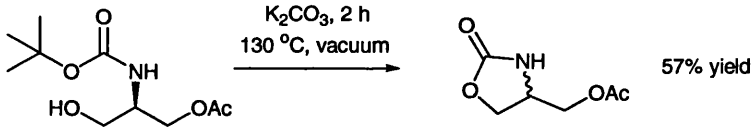


(*R*)-3.2 enantiomerically enriched **3.3**

Scheme 3.12. Cyclisation of acetate **3.2**

3.4.1 Synthesis of racemic 4-acetoxymethyl-2-oxazolidinone and kinetic resolution by enzymatic hydrolysis

The intramolecular reaction was first attempted under basic conditions. Treatment of enantiomerically enriched acetate **3.2** with potassium carbonate at 130 °C under vacuum[30] afforded the racemic oxazolidinone **3.3**(Scheme 3.13).

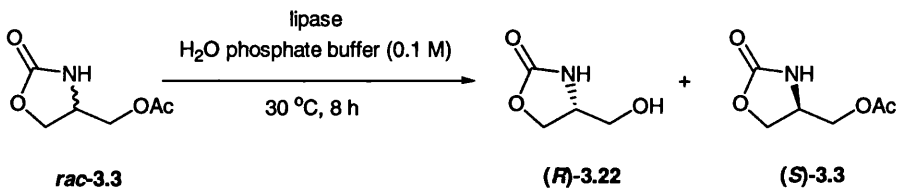


(*R*)-3.2 *rac*-3.3

Scheme 3.13. Synthesis of racemic 4-acetoxymethyl-2-oxazolidinone **3.3**

Unfortunately, this product was obtained as a racemic mixture. We assume that intramolecular acetyl transfer occurs prior to the cyclisation, thereby affording the racemic product.

Nevertheless, kinetic resolution of ***rac*-3.3** was achieved by enzymatic hydrolysis in phosphate buffer (Scheme 3.14).



Scheme 3.14. Lipase-catalysed kinetic resolution of ***rac*-3.3**

Porcine pancreas lipase, lipase from *Pseudomonas cepacia* and from *Candida antarctica* type B were examined for their activity and selectivity towards the reaction studied.

The reactions were stopped after 8 hours by removal of the enzymes by filtration. The yields of the reactions catalysed by the different lipases were determined after chromatographic purification of the products. The optical purity of hydroxymethyl oxazolidinone **(R)-3.4** was determined comparing the specific rotation of the obtained alcohol with that reported in literature for the corresponding enantiopure form.[31,32] The results are illustrated in Table 3.4.

Table 3.4. Kinetic resolution of ***rac*-3.3** with different lipases[a]

Entry	Lipase	Yield (%)	Optical purity (%)[b]
1	CAL B	33	93
2	PCL	36	90
4	PPL	42	84

[a] 140 $mg_{enzyme}/mmol_{substrate}$ in 1.5 mL phoshate buffer solution (0.1 M, pH 7) using 0.6 mmol of racemic 4-acetoxymethyl-2-oxazolidinone **3.3**.

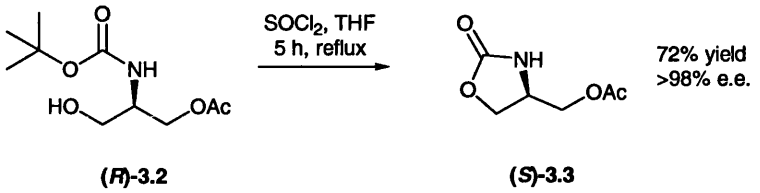
[b] optical purity (%) = $\{[\alpha]^{D}_{30}$ of obtained (*R*)-4} / { $[\alpha]_{D}^{30}$ of enantiopure (*R*)-4} x 100.

The (*R*)-enantiomer of oxazolidinone **3.3** reacted faster than the (*S*)-enantiomer. After 8 hours, optical purities greater than 84% were detected.

3.4.2 Synthesis of enantiomerically enriched (*S*)-4-acetoxymethyl-2-oxazolidinone

We decided to try another approach to the cyclisation reaction reported in Scheme 3.13 and to avoid basic conditions.

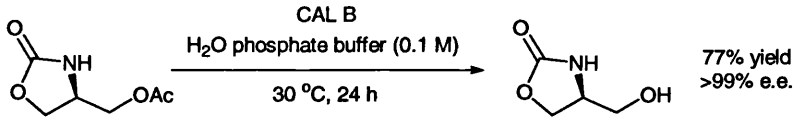
Enantiomerically enriched (*S*)-oxazolidinone **3.3** was obtained in a one-step reaction by cyclisation with thionyl chloride (Scheme 3.15).[33,34]



(*R*)-3.2 (*S*)-3.3

Scheme 3.15. Synthesis of (*S*)-4-acetoxymethyl-2-oxazolidinone **3.3**

The reaction proceeded with >98% enantiomeric excess and 72% yield.
The absolute stereochemistry of oxazolidinone **(*S*)-3.3** was confirmed by enzymatic hydrolysis of the acetate group (Scheme 3.16) and comparison of the specific rotation of hydroxy oxazolidinone **3.22** with literature data.[31,32]



(*S*)-3.3 (*S*)-3.22

Scheme 3.16. Hydrolysis of 4-acetoxymethyl-2-oxazolidinone

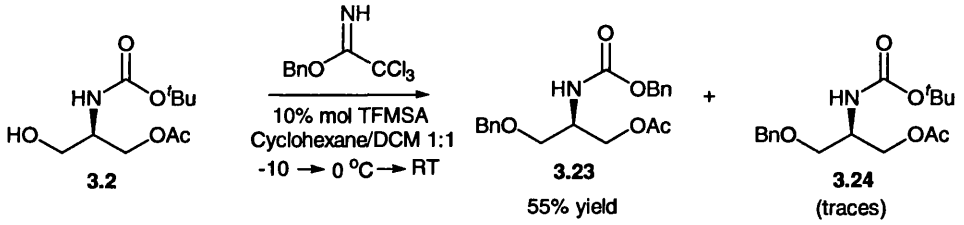
The synthetic utility of 4-hydroxymethyl-2-oxazolidonone **3.22** has previously been demonstrated in its straightforward conversion into 4-benzyl and 4-ethyl derivatives.[31] We can note that *Candida antarctica* lipase type B preferred the (*R*)-enantiomer when it had a choice of the two enantiomers (Scheme 3.14). However, the same enzyme hydrolysed the (*S*)-enantiomer, in the absence of the other enantiomer (Scheme 3.16).

3.4.3 Synthesis of enantiomerically enriched (*R*)-4-benzyloxymethyl-2-oxazolidinone

The acetoxy group of oxazolidinone **3.3** and the hydroxy group of oxazolidinone **3.22** represent a limitation to the use of these enantiomerically enriched oxazolidinones in chiral auxiliary-based chemistry, where strong basic conditions are often required.

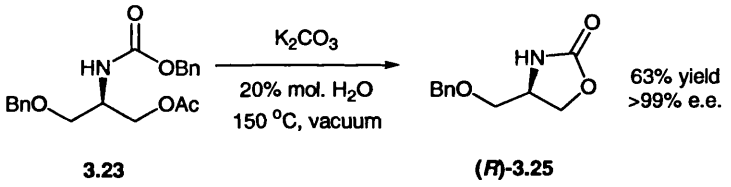
Though they can be easily converted into their 4-alkyl substituted forms,[31] we envisaged that protection of the oxygen would be an advantage.

In an alternative approach, the enantiomerically enriched mono-acetate **3.2** was benzylated using benzyl trichloroacetimidate, in the presence of a catalytic amount of triflic acid,[35-37] to afford the *N*-benzyloxycarbonyl-*O*-benzyl acylated serinol **3.23** in reasonable yield. Traces of the analogue *N*-Boc-*O*-benzyl acylated serinol **3.24** were also detected during the reaction (Scheme 3.17).



Scheme 3.17. Protection of the hydroxylic group

Cyclisation with potassium carbonate[30] then provided the (*R*)-oxazolidinone **3.25** in > 99% e.e. (Scheme 3.18).



Scheme 3.18. Synthesis of enantiomerically enriched 4-benzyloxymethyl-2-oxazolidinone

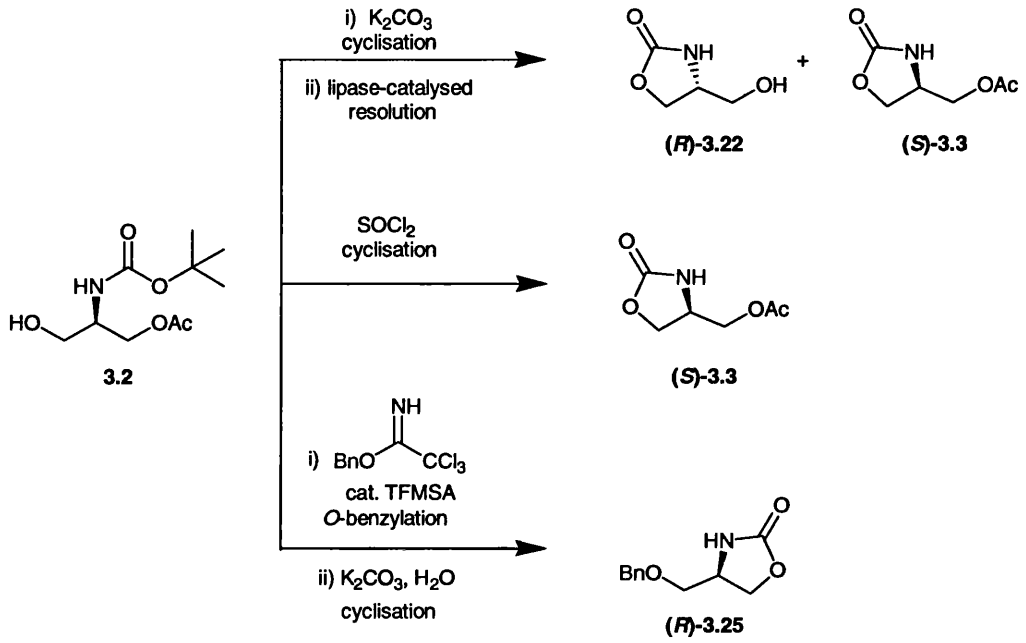
Protection of the hydroxylic group avoided any racemisation during the base-catalysed cyclisation reaction. Catalytic water was found to be essential for the activation of the acylated hydroxy group as nucleophile.

3.5 Conclusions

Different lipases have been shown to catalyse selectively the mono-acetylation of *N*-Boc-serinol with vinyl acetate in organic solvent. Aspects of the reaction such as effect of solvent, temperature and lipase origin were discussed and we can conclude

that the desymmetrisation of *N*-Boc-protected serinol can be successfully achieved in good yield and high e.e. in 2 hours, by using the inexpensive crude commercial PPL in vinyl acetate at 30 °C.

The desymmetrisation of *N*-Boc-serinol has been exploited in different ways to prepare enantiomerically enriched (4*S*)- and (4*R*)-substituted 2-oxazolidinones, as illustrated in Scheme 3.19.[38]



Scheme 3.19. Preparation of 2-oxazolidinones by enzymatic desymmetrisation

3.6 References

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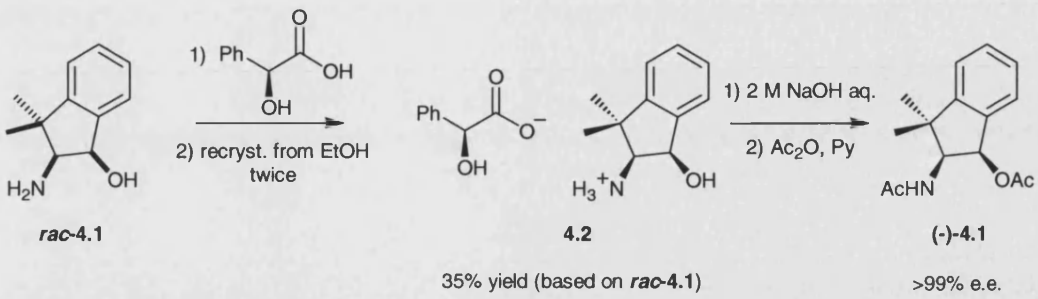
4. Enzymatic Kinetic Resolution of Racemic Evans' Auxiliaries

4.1 Introduction

Since 1981, when Evans introduced the use of enantiomerically pure 2-oxazolidinones (Evans' auxiliaries) to achieve asymmetric syntheses,[1] the development of chiral auxiliaries and reagents has been a matter of utmost interest.
Most chiral auxiliaries are derived from naturally occurring homochiral compounds and their derivatives,[2] but limitations in their structural modifications and lack of accessibility to their configurational antipodes are sometimes an obstacle in improvement of the selectivity of asymmetric reactions. Therefore, the development of new enantiomerically pure compounds, which can be suitably designed for each asymmetric process, has recently received considerable attention and some successful examples have been reported.[3-12]
Nevertheless, chiral auxiliaries used in asymmetric transformations need to be enantiomerically pure. This makes them expensive and difficult to prepare. Especially considering the paradox that the main source of the most efficient chiral auxiliaries, 2-oxazolidinones, are chiral α-amino alcohols, and their asymmetric synthesis sometimes, in turn, is achieved using chiral auxiliaries.[13-15]
This explains why the preparation of chiral auxiliaries by either resolution of the racemic precursor or by direct resolution of chemically designed racemic auxiliaries has received increasing attention.

4.2 Background

Resolution of suitably designed racemic α-amino alcohols is one of the main methods used to synthesise chiral Evans' auxiliaries.
As an example, resolution of racemic *cis*-2-amino-3,3-dimethyl-1-indanol **4.1** by selective crystallisation of the diastereoisomeric salts **4.2** with (*S*)-mandelic acid has been reported (Scheme 4.1).[16]

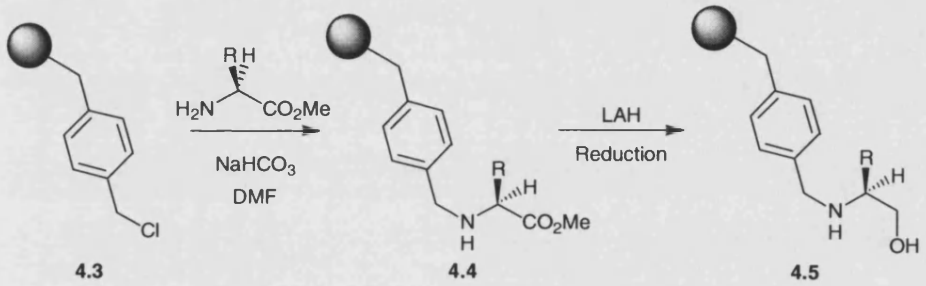


***rac*-4.1** **4.2** **(-)-4.1**

35% yield (based on ***rac*-4.1**) >99% e.e.

Scheme 4.1. Resolution of a racemic indanol derivative

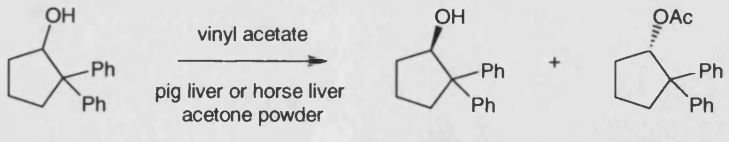
Solid phase parallel synthesis starting from Merrifield resins can be very useful for the preparation of a variety of amino alcohols (Scheme 4.2).[17]



4.3 **4.4** **4.5**

Scheme 4.2. Solid phase synthesis of enantiomerically enriched amino alcohols

However, in the last few years there have been seen several examples of the direct design of racemic auxiliaries and their enzymatic resolution.
2,2-Diphenylcyclopentanol **4.6**, an efficient chiral auxiliary, and structurally related hindered secondary alcohols were resolved by enantioselective hydrolysis of their acetate esters with either pig liver or horse liver acetone powder (Scheme 4.3).[18]

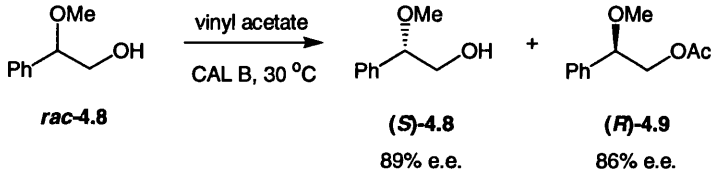


***rac*-4.6** **(*R*)-4.6** **(*S*)-4.7**

96% e.e. 92% e.e.

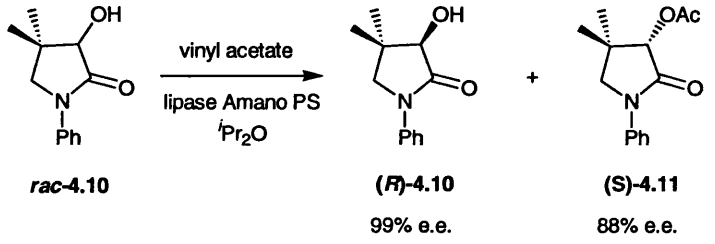
Scheme 4.3. Enzymatic resolution of 2,2-diphenylcyclopentanol **4.6**

Enzymatic resolution of the chiral auxiliary 2-methoxy-2-phenylethanol **4.8** has also been achieved *via* several lipase-catalysed processes.[19] An example is reported in Scheme 4.4.



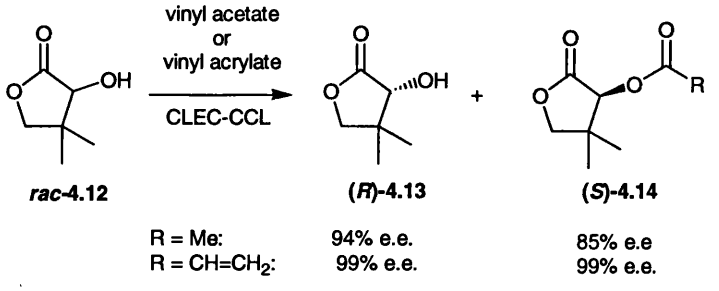
Scheme 4.4. Enzymatic resolution of 2-methoxy-2-phenylethanol

Lipase-catalysed resolution of a "pantolactone type" racemic auxiliary **4.10** afforded resolved D- and L- pantolactone analogues **(*R*)-4.10** and **(*S*)-4.11**. (Scheme 4.5).[20]



Scheme 4.5. Lipase-catalysed resolution of a pantolactone analogue racemic auxiliary

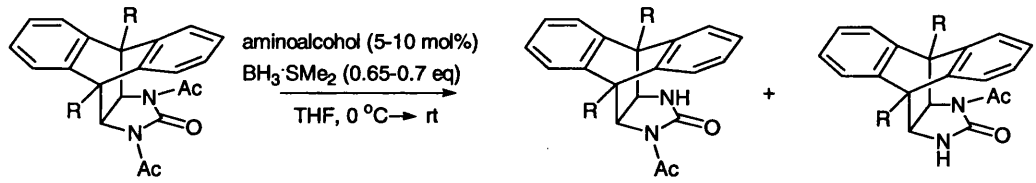
Moreover, Williams and co-workers have reported recently that racemic pantolactone **4.12** can be converted into either enantiomerically enriched pantolactone acetate or pantolactone acrylate **4.14** by an enzyme-catalysed kinetic resolution process (Scheme 4.6).[21]



Scheme 4.6. Lipase-catalysed resolution of pantolactone

Enantiomerically enriched 2-oxazolidinones have also been prepared by resolution. Though chromatographic separation is widely used to resolve enantiomers,[22] chemical and enzymatic resolutions still remain the most used methods. Herein we wish to report some examples.

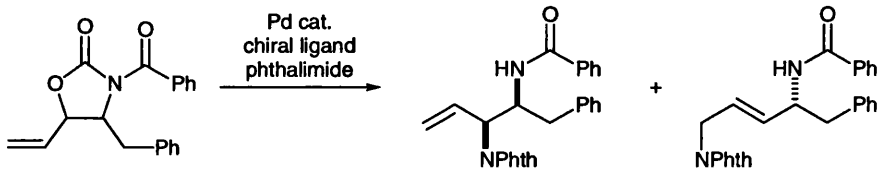
A reductive monodeacetylation, catalysed by oxazaborolidines derived from conformationally rigid chiral aminoalcohols is successfully applied to the kinetic resolution of racemic 1-acetyl-2-oxazolidinones (Scheme 4.7).[23]



4.15 **4.16** >98% yield **4.16**

Scheme 4.7. Kinetic resolution of oxazolidinones by reductive monodeacetylation

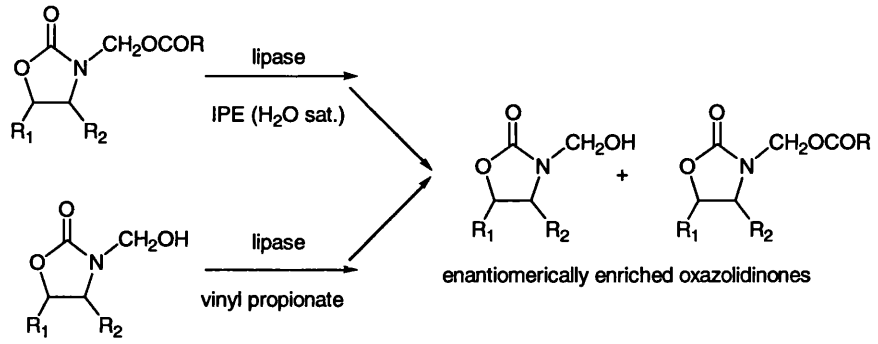
A regiodivergent kinetic resolution of racemic 5-vinyloxazolidinones **4.17** by allylic substitution with phthalimide and a palladium catalyst to afford optically enriched regioisomers products has been recently reported, as illustrated in Scheme 4.8.[24]



***rac*-4.17** **4.18** 5 : **4.19** 1

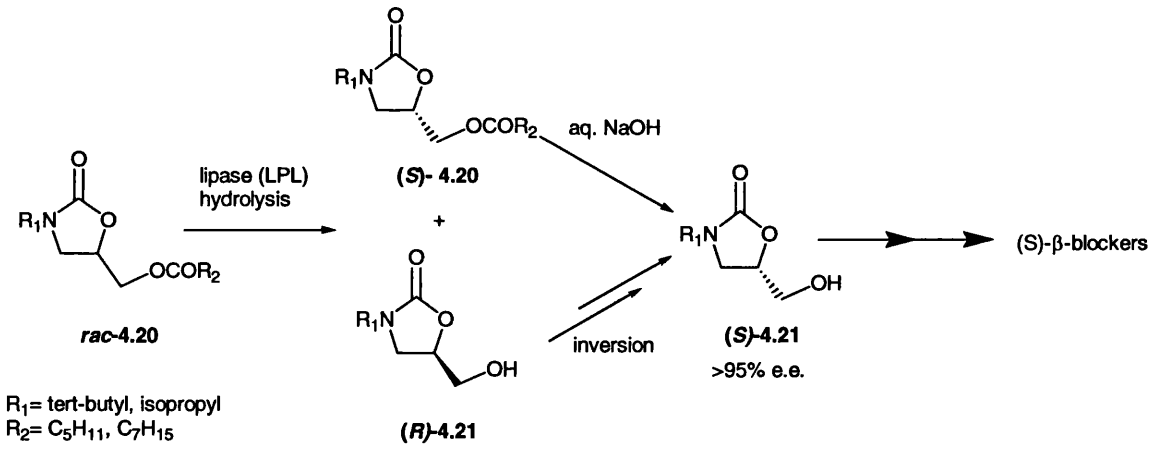
Scheme 4.8. Regiodivergent kinetic resolution of racemic 5-vinyloxazolidinones

Enantiomerically pure 4- or 5- substituted 2-oxazolidinones were obtained by lipase-catalysed enantioselective hydrolysis of the 3-acyloxymethyl-2-oxazolidinones and transesterification of the 3-hydroxymethyl- 2-oxazolidinones with vinyl propionate in organic solvent (Scheme 4.9).[25]



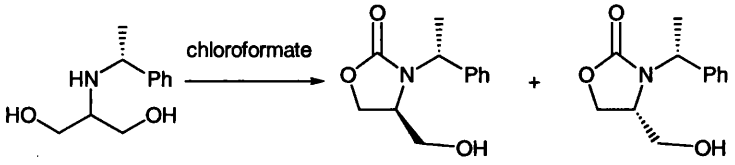
Scheme 4.9.Lipase-catalysed resolution of 3-acyloxymethyl and 3-hydroxymethyl -2-oxazolidinones

Hydrophobic (*R,S*)-5-acyloxymethyl-3-alkyl-2-oxazolidinones were successfully hydrolysed stereoselectively with lipoprotein lipase Amano 3 (LPL) adsorbed on Amberlite, as shown in Scheme 4.10.[26]



Scheme 4.10. Enzymatic selective hydrolysis

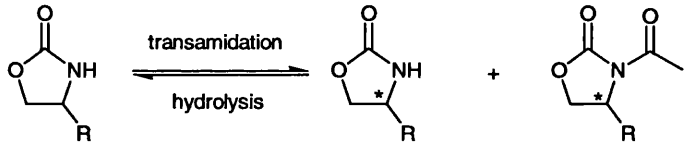
Asymmetric desymmetrisation of 2-[(α*R*)-α-methyl benzyl] amino-1, 3-propanediol **4.22** with 2-chloroformate and DBU gave diastereomerically enriched (4*S*)-4-hydroxymethyl-N- [(αR)-α-methylbenzyl]-2-oxazolidinone **(4*S*)-4.23** (Scheme 4.11).[27]



4.22 **(4*S*)-4.23** **(4*R*)-4.23**
>90% d.e.

Scheme 4.11. Desymmetrisation of 2-[(α*R*)-α-methyl benzyl] amino-1, 3-propanediol

After these examples and the successful enzymatic resolution of racemic pantolactone, we envisaged that an enzymatic reaction could also be used to resolve racemic Evans' auxiliary oxazolidinone. Under suitable conditions, we believed that the resolution of racemic Evans' auxiliaries could be accomplished by either an enzyme-catalysed transamidation reaction or by hydrolysis of the amide derivative (Scheme 4.12).



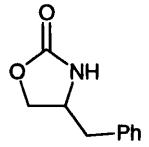
racemic

Scheme 4.12. General approach to the enzymatic resolution of Evans' auxiliaries

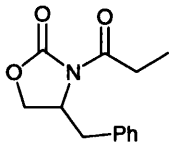
The same reaction would also be a simple alternative method to accomplish attachment/detachment of an achiral substrate to a chiral auxiliary.

4.3 The attempted enzymatic kinetic resolution of chiral Evans' auxiliaries

The main priority was to employ an Evans' auxiliary previously used in asymmetric synthesis and known to activate the substrate toward reaction. For this reason, the Evans' auxiliary 4-benzyl-2-oxazolidinone[28] was chosen as the racemic auxiliary for the transamidation reaction in organic media. In addition, the commercially available acylated form, 4-benzyl-3-propionyl-2-oxazolidinone was tested in the hydrolysis reaction (Scheme 4.13).



4.24
4-benzyl-2-oxazolidinone



4.25
4-benzyl-3-propionyl-2-oxazolidinone

Scheme 4.13

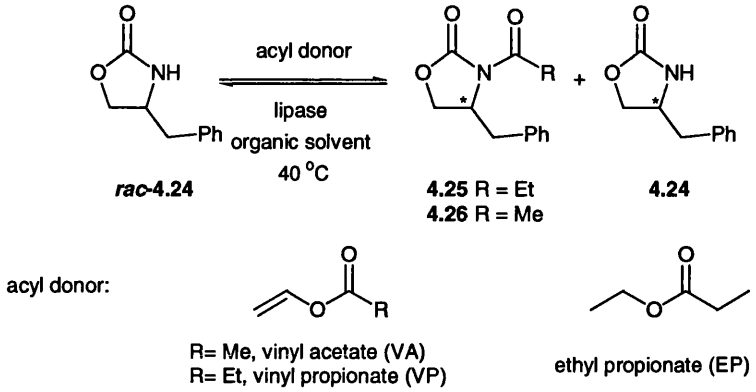
The enzymes chosen had proved to be the most selective and active in transamidation and amide hydrolysis reactions.[29-34] Lipases from *Candida antarctica*, *Candida cylindracea*, *Pseudomonas fluorescens* and from *Mucor javanicus* were tested in the kinetic resolution of the 4-benzyl-2-oxazolidinone. Lipases from *Candida antarctica* and from *Candida cylindracea*, protease from *Aspergillus oryzae*, *Penicillum Amidase*, proteinase from *Bacillus subtilis* and esterase from hog liver were tested in the kinetic resolution of the acylated 4-benzyl-2-oxazolidinone *via* the hydrolysis reaction.

Vinyl acetate, vinyl propionate and ethyl propionate were employed as acylating agents. Vinyl esters are often used as acyl donors, because the resulting vinyl alcohols are rapidly converted into the corresponding aldehydes, thus making the transesterification or transamidation irreversible.[35-38]

As the influence of the reaction medium on both catalytic activity and stereoselectivity in lipase catalysed kinetic resolutions is well documented in the literature,[39-41] the same reaction was also performed in different organic solvents or aqueous buffers.

4.3.1 4-Benzyl-2-oxazolidinone enzymatic *N*-acylation

The enzymatic resolution of (*R/S*)-(+/-)-4-benzyl-2-oxazolidinone *via N*-acylation was studied in organic solution at a standard temperature of 40 °C (Scheme 4.14).



Scheme 4.14. Lipase-catalysed *N*-acylation of *rac*-4-benzyl-2-oxazolidinone

Four lipases (lipase from *Candida antarctica* type B, lipase from *Pseudomonas fluorescens* immobilised in Sol-Gel-AK, lipase from *Mucor javanicus* and lipase from *Candida cylindracea*) were tested for their activity and selectivity towards the acylation reaction.

Three different acylating agents were examined (vinyl acetate, vinyl propionate and ethyl propionate), and the substrate/acylating agent ratio was varied from 1:1 up to 1:100.

The same reaction was also performed in reaction media with different hydrophobicity, functionality and structure. Diisopropyl ether, *n*-hexane, dioxane, toluene, vinyl acetate, vinyl propionate, diisopropyl ether/water, *n*-hexane/water and *n*-hexane/isopropanol were all investigated.

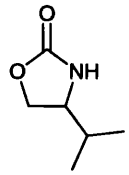
These results are comprehensively tabulated in Table 4.1.

Table 4.1. Variations in the lipase-catalysed *N*-acylation of *rac*-4-benzyl-2-oxazolidinone

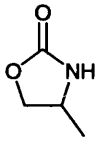
Substrate (mmol)	Substrate conc. (M)	Acyl donor	Substrate/ acyl donor	Solvent	Enzyme ($mg_{enz}/mmol_{sub}$)	Time (h)	Conv. (%)
0.05	0.05	EP	1:1	$^{i}Pr_2O$	CCL, 400	48	0
0.02	0.02	EP	1:1	$^{i}Pr_2O$	CCL, 400	48	0
0.05	0.05	EP	1:1	$^{i}Pr_2O$	CAL B, 400	48	0
0.01	0.01	EP	1:1	$^{i}Pr_2O$	CAL B, 400	72	0
0.01	0.01	VP	1:1	$^{i}Pr_2O$	CAL B, 400	72	0
0.25	0.05	EP	1:1	$^{i}Pr_2O$	CCL, 80	48	0
0.25	0.05	EP	1:1	dioxane	CCL, 400	66	0
0.25	0.05	VP	1:1	dioxane	CCL, 400	66	0
0.25	0.05	VP	1:1	*n*-hexane	CCL, 400	66	0
0.25	0.05	VA	1:100	dioxane	CCL, 400	72	0
0.25	0.05	VP	1:100	dioxane	CCL, 400	72	0
0.25	0.05	VP	1:100	*n*-hexane	CCL, 160	66	0
0.1	0.05	EP	1:1	$^{i}Pr_2O/H_2O$ 98:2	CCL, 400	72	0
0.1	0.05	VP	1:1	*n*-hexane	PFL, 700	72	0
0.1	0.05	EP	1:1	$^{i}Pr_2O$	MJL, 450	72	0
0.2	0.05	VP	-	VP	CCL, 500	96	0
0.2	0.1	EP	1:1	$^{i}Pr_2O$	CAL B, 200	72	0
0.2	0.1	VP	1:10	*n*-hex./IPA 90:10	CAL B, 200	72	0
0.4	0.4	VP	-	VP	CAL B, 38	48	0
0.3	0.3	VA	-	VA	CAL B, 50	48	0
0.4	0.4	VP	1:4	toluene	CAL B, 38	48	0
0.3	0.3	EP	1:4	toluene	CAL B, 50	48	0
0.1	0.1	VP	1:3	*n*-hexane (H_2O sat.)	CAL B, 200	96	0
0.3	0.3	VA	-	VA	CAL B, 3	24	0
0.3	0.3	VP	-	VP	CAL B, 3	24	0

In all cases, after 2-4 days of reaction time no enzyme activity (the reactions were followed by chiral HPLC analysis) was observed.

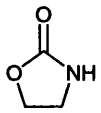
Rationalising these results, it was conceivable that the substrate chosen was too sterically demanding. Therefore, a more hindered example such as 4-isopropyl-2-oxazolidinone and less hindered examples such 4-methyl-2-oxazolidinone and 2-oxazolidinone were also investigated (Scheme 4.15).



4.27
4-isopropyl-2-oxazolidinone



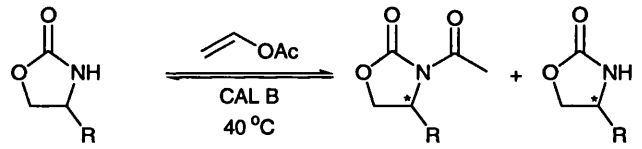
4.28
4-methyl-2-oxazolidinone



4.29
2-oxazolidinone

Scheme 4.15. Less hindered racemic auxiliaries

4-Isopropyl-2-oxazolidinone ***rac*-4.27**, 4-methyl-2-oxazolidinone ***rac*-4.28** and 2-oxazolidinone **4.29** were examined in the *N*-acylation reaction catalysed by *Candida antarctica* lipase B. These reactions were performed in vinyl acetate at 40 °C (Scheme 4.16).



***rac*-4.27** R = ^{i}Pr
***rac*-4.28** R = Me
4.29 R = H

4.30 R = ^{i}Pr
4.31 R = Me
4.32 R = H

4.27 R = ^{i}Pr
4.28 R = Me
4.29 R = H

Scheme 4.16. Lipase-catalysed *N*-acylation of *rac*-4-isopropyl/methyl-2-oxazolidinone

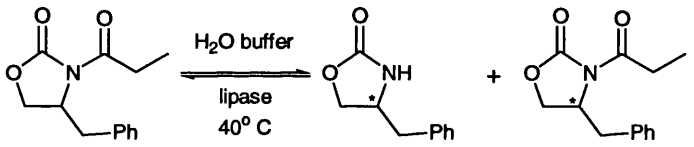
Every reaction was carried out at 0.3 M concentration in vinyl acetate solution and followed for 24 h. Different enzyme concentrations were tested (Table 4.2). Still no reaction was observed after 24 hours reaction time.

Table 4.2. *N*-acylation of other 2-oxazolidinones

Substrate (mmol)	Acyl donor and solvent	Enzyme ($mg_{enz}/mmol_{sub}$)	Time (h)	Conv. (%)
4-isopropyl-2-oxazolidinone 0.3	VA, 1 ml	33	24	0
4-isopropyl-2-oxazolidinone 0.3	VA, 1 ml	67	24	0
4-isopropyl-2-oxazolidinone 0.3	VA, 1 ml	100	24	0
4-methyl-2-oxazolidinone 0.3	VA, 1 ml	33	24	0
2-oxazolidinone 0.3	VA, 1 ml	167	24	0

4.3.2 3-Propionyl-4-benzyl-2-oxazolidinone enzymatic hydrolysis

The next stage of our investigation concerned the enzymatic resolution of racemic 4-benzyl-3-propionyl-2-oxazolidinone **4.25**. Hydrolysis in an aqueous buffer, at 40 °C was performed as indicated in Scheme 4.17.



rac-4.25 4.24 4.25

Scheme 4.17. Lipase-catalysed hydrolysis of *rac*-4-benzyl-3-propionyl-2-oxazolidinone

The lipase from *Candida antarctica* type B and the lipase from *Candida cylindracea* were tested for their activity and selectivity in two different aqueous buffers, both at pH 7.2 (Table 4.3).

Table 4.3. Variations in the lipase-catalysed hydrolysis of *rac*-4-benzyl-3-propionyl-2-oxazolidinone

Substrate (mmol)	**Solvent**	**Enzyme (g_{enz}/$mmol_{sub}$)**	**Time (h)**	**Conv. (%)**
0.02	tris/HCl buffer, 2 ml 5 mM, pH 7.2	CCL 1.5 g/$mmol_{sub}$	72	0
0.02	hepes/HCl, 2 ml 10mM, pH 7.2	CAL B 1.5 g/$mmol_{sub}$	72	0
0.02	tris/HCl buffer, 2 ml 5 mM, pH 7.2	CCL 1.5 g/$mmol_{sub}$	72	0
0.02	hepes/HCl, 2 ml 10mM, pH 7. 2	CAL B 1.5 g/$mmol_{sub}$	72	0

No enzymatic reaction was observed. After 72 h, the same extent of conversion, followed by HPLC analysis, was observed in absence (control) or presence of the catalyst.

In the absence of success with lipase enzymes, we then turned our attention to hydrolases. Protease from *Aspergillus oryzae,* proteinase from *Bacillus subtilis, Penicillum* amidase from *Escherichia coli,* esterase from hog liver (immobilised on Eupergit C) were investigated for their activity and selectivity in phosphate buffer (Table 4.4).

Table 4.4. 3-Propionyl-4-benzyl-2-oxazolidinone hydrolysis catalysed by other hydrolases

Substrate (mmol)	Solvent	Enzyme ($mg_{enz}/mmol_{sub}$)	Time (h)	Conv. (%)
0.3	phosphate buffer 0.01 M, pH 7, 1 ml	Protease from *Aspergillus o.* 150 $mg/mmol_{sub}$	48	0
0.3	phosphate buffer 0.01 M, pH 7, 1 ml	*Penicillum* Amidase 133 $mg/mmol_{sub}$	48	0
0.3	phosphate buffer 0.01 M, pH 7.5, 1 ml	Proteinase from *Bacillus s.* 67 $mg/mmol_{sub}$	48	0
0.3	phosphate buffer 0.01 M, pH 7.5, 1 ml	Proteinase from *Bacillus s.* var. Biotech. A, 67 $mg/mmol_{sub}$	48	0
0.3	phosphate buffer 0.01 M, pH 8, 1 ml	Esterase from hog liver imm. on Eupergit, 67 $mg/mmol_{sub}$	48	0

Despite these attempts, no reaction was observed after 48 h using different enzymes as catalysts.

4.4 Conclusions

We attempted the enzymatic kinetic resolution of different 4-substituted 2-oxazolidinones, by either *N*-acylation of hydrolysis of the substrate. Though we tested a range of enzymes in different reaction conditions, no enzymatic reaction was observed.

From these results detailed here, it seemed to be evident that the screened enzymes were not active towards the desired reactions.

Lipases are known to have a very selective catalytic reaction site,[42-44] and though the utility of lipases in *N*-acylation reactions for the preparation of chiral amides from primary amines has been demonstrated demonstrated,[45-55] the resolution of secondary amines/amides via enzyme-catalysed acylation/hydrolysis is a relative rare process.[56-58] In this respect, the observed results are perhaps unsurprising.

4.5 References

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5. Racemic Auxiliaries: Applications to Asymmetric Synthesis

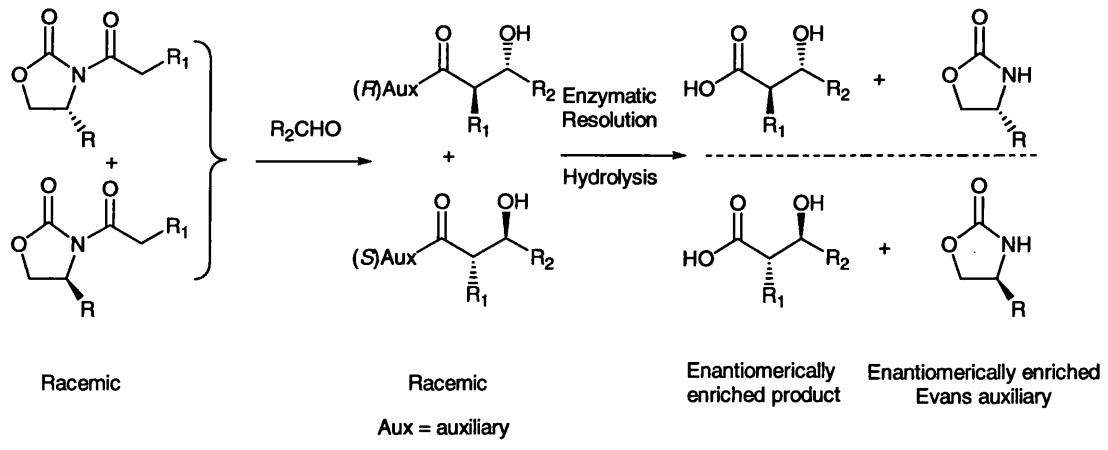
5.1 Introduction

For a selective catalyst to be generally useful, it must combine two opposing qualities. It must accept a broad range of substrates; yet also retain a high selectivity for each one. Lipases are among the few catalysts that fulfil both criteria[1] and that is the reason why they are so widely used in stereoselective catalysis.

Nevertheless, lipase and enzyme reactivity in general can hardly be improved. Either they are active towards the studied reaction, with general high activity and stereoselectivity, or they are not active at all. That was actually what we experienced in our attempt of enzyme-catalysed kinetic resolution of racemic Evans' auxiliaries (see previous chapter).

However, the proposal for the use of racemic auxiliaries to achieve asymmetric synthesis still seemed to be a valid idea.

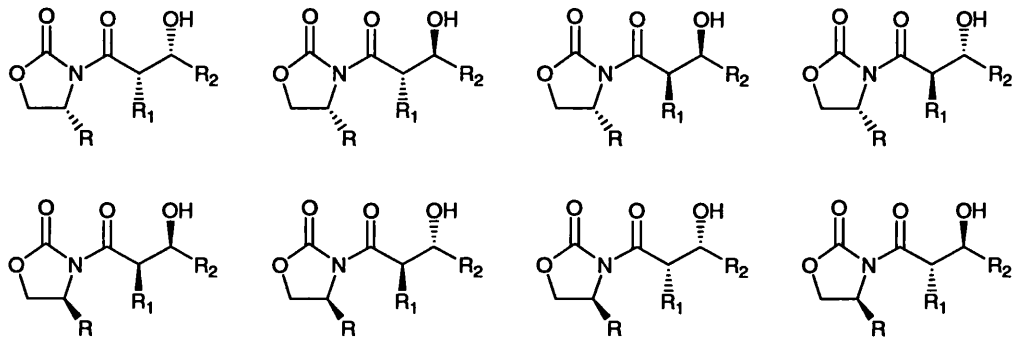
We decided to attempt an unusual approach to asymmetric synthesis, using racemic aldol adduct from a diastereoselective aldol reaction and an enzymatic resolution. In order to achieve this goal, the auxiliary has to relay its stereochemistry to the substrate, which can then be resolved by the enzyme (Scheme 5.1).



Scheme 5.1. General approach to asymmetric synthesis with a racemic auxiliary

We envisaged that through the application of this methodology we could eventually obtain enantiomerically enriched auxiliary and enantiomerically enriched product.

The main objective of the overall process was to find a good stereoselective aldol reaction, which could afford only two enantiomers, separable by an enzyme-catalysed resolution.
To understand the difficulty of achieving this goal, it has to be considered that eight possible stereoisomers can be obtained in the aldol reaction between a racemic acylated oxazolidinone and an aldehyde. This is illustrated in Scheme 5.2.



Scheme 5.2. Possible isomers from an aldol reaction with racemic auxiliary

The actual number of isomers formed depends upon the stereoselectivity of the aldol reaction used.

5.2 Background. Asymmetric aldol reactions with Evans' chiral auxiliaries

The literature on stereocontrolled aldol reactions using chiral auxiliaries was very useful to our investigation. Evans' chiral auxiliaries provide good stereocontrol in aldol reactions. Much of the work on asymmetric aldol reactions has actually concentrated on the development of chiral auxiliaries, which control the diastereoselectivity and the enantioselectivity of the enolates.[2] The stereochemistry of the enolate has an important bearing on the stereoselectivity of the subsequent aldol reactions, along with several other variables including the nature of the aldehyde, the metal, the solvent and the precise reaction conditions.

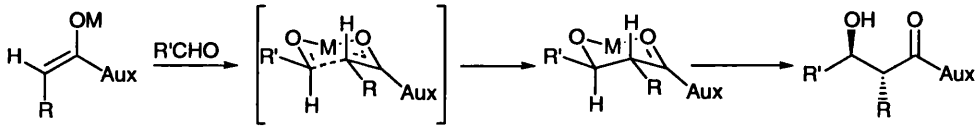
Given all these variables it might be expected that "rules" could be elaborated for the prediction of the outcome of an aldol reaction. Fortunately, a great body of work already exists and from this it is often possible to make a reasonable prediction in most cases.

The stereochemical outcome of the aldol reaction can be discussed by using the chair-like Zimmerman-Traxler transition-state model.[3] In this model, the aldehyde associates with the enolate-metal complex, and the aldol reaction proceeds *via* a six-membered transition state.

For many enolates it is found that the *E*-isomer gives an *anti* aldol product, and the *Z*-isomer a *syn* aldol product (Scheme 5.3).

(*E*)-Enolate

Bonding from the lower face of the enolate

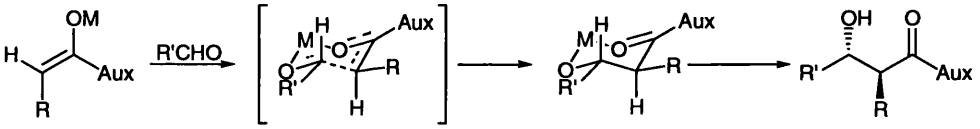


R "equatorial"
R' "equatorial"

(2*R*,3*S*)-*anti*

(*E*)-Enolate

Bonding from the upper face of the enolate

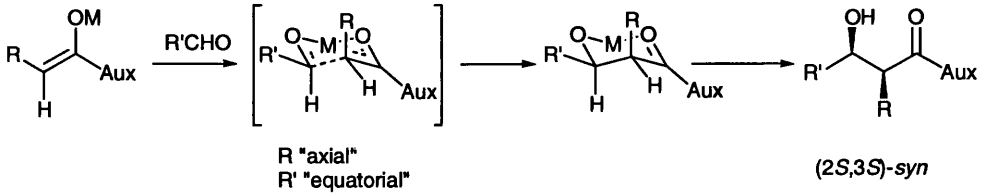


R "equatorial"
R' "equatorial"

(2*S*,3*R*)-*anti*

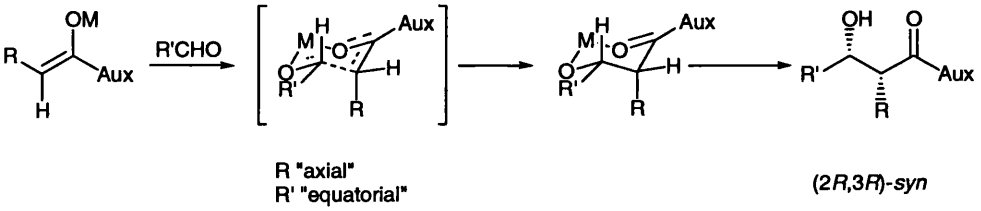
(*Z*)-Enolate

Bonding from the lower face of the enolate



(*Z*)-Enolate

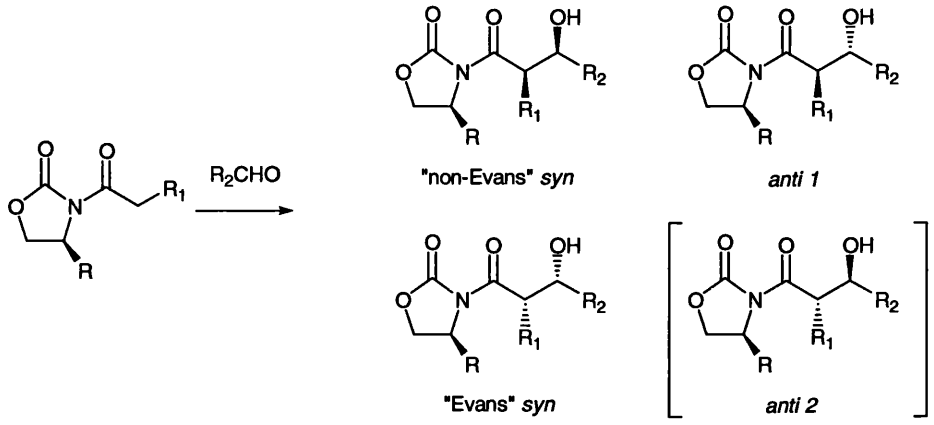
Bonding from the upper face of the enolate



Scheme 5.3. Stereochemistry of a chiral auxiliary-based aldol reaction

Which of the two possible *anti* or *syn* aldol products is obtained will depend on which face of the enolate reacts preferentially, which in turn usually depends on the chiral auxiliary (Aux).

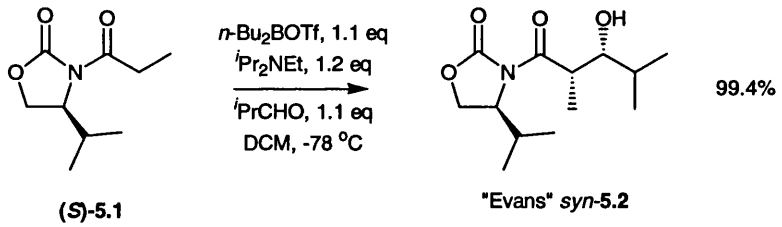
Chiral auxiliaries are likely to be sterically demanding and favour the formation of *Z*-enolates, and therefore it is not difficult to understand why *syn* selective asymmetric aldol reactions are much more common than those leading to *anti* aldol products. This means that among the four possible isomers from an aldol reaction with a chiral Evans' auxiliary, the two *syn* "Evans" and "non-Evans" aldol adducts are likely to be preferentially formed. Sometimes the *anti 1* is also formed; no formation of the other *anti* has ever been reported (Scheme 5.4).



Scheme 5.4. Possible isomers from an aldol reaction with chiral auxiliary

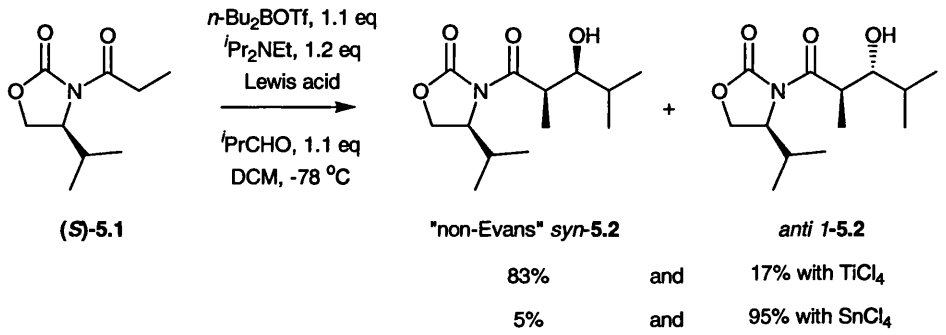
Through these general "rules", the attributes of the aldol reaction can be improved by the introduction of architecturally refined enolate metal centres. Herein a few examples essential to our study are reported (*vide supra*).

The most frequently used procedure utilises boron-enolates. The *N*-propionyl-oxazolidinone **5.1** undergoes a highly stereoselective enolisation with di-*n*-butylboron triflate (Scheme 5.5).[4] The observed diastereomer ratio "Evans" *syn*/other diastereomers is 99.4:0.6, in favour of the "Evans" *syn* aldol product **5.2**.



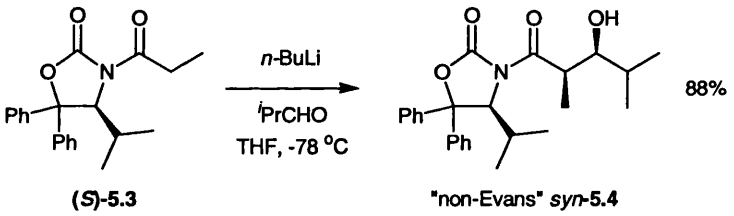
Scheme 5.5. The aldol reaction *via* B-enolate

When the same Evans' reagent is reacted with isobutyraldehyde under Lewis acid catalysis, the major product is either the "non-Evans" *syn* aldol **5.2** or *anti 1* aldol **5.2**, depending on the Lewis acid used (Scheme 5.6).[5]



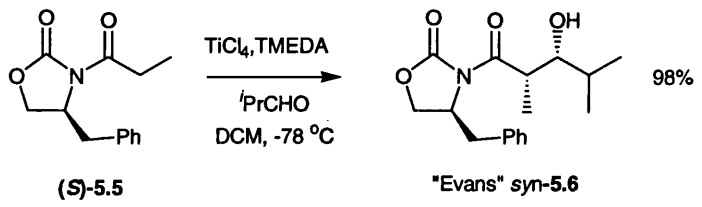
Scheme 5.6. The aldol reaction *via* B-enolate under Lewis-acid catalysis

The Li-enolate of *N*-propyl-oxazolidinone **5.3** undergoes a moderately stereoselective aldol reaction with isobutyraldehyde giving the "non-Evans" *syn* aldol **5.4** as major product (Scheme 5.7).[6]



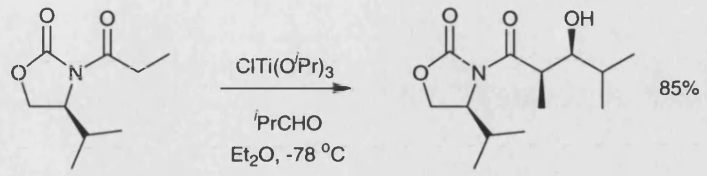
Scheme 5.7. The aldol reaction *via* Li-enolate

The tetrachlorotitanium-enolate of 3-propionyl-4-benzyl-2-oxazolidinone **5.5** gave the same diastereoselection reported for the analogous boron-mediated processes, when reacted with isobutyraldehyde in the presence of tetramethylethylenediamine (Scheme 5.8).[7]



Scheme 5.8. The aldol reaction *via* $TiCl_4$-enolate

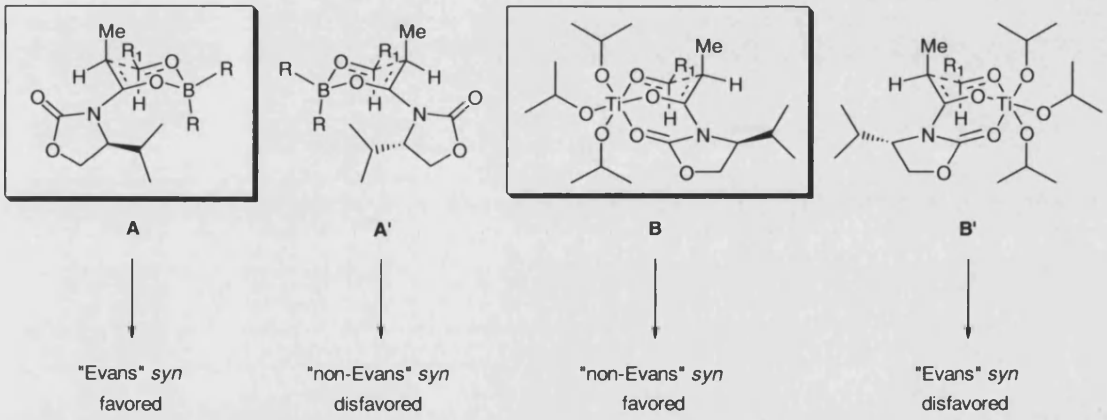
Whereas $(^{i}PrO)_3TiCl$-mediated aldol reactions give a reversal of diastereofacial selectivity, being the "non-Evans" *syn* aldol **5.2** the major product (Scheme 5.9).[8]



(*S*)-5.1 "non-Evans" *syn*-**5.2**

Scheme 5.9. The aldol reaction *via* Ti –enolate from $(^{i}PrCHO)_3TiCl$

The stereochemical outcome of the aldol reactions using different metal-enolates can be rationalised by considering the chair-like transition-state model. Thus, boron-enolate derivatives produce "Evans" *syn* aldols *via* intermediate **A**, and lithium- or titanium-enolates are thought to use an additional co-ordination site, to give "non-Evans" *syn* aldols *via* **B** (Scheme 5.10).[5,8]



Scheme 5.10. Stereochemical outcome of B- and Ti-enolate mediated aldol reaction

Use of a titanium enolate would enable chelation control, while at the same time the presence of ligands on the Ti would enhance the selectivity.[9-10] Boron has given high levels of stereocontrol in aldol reactions, where the presence of added ligands is believed to be the controlling factor. However boron is incapable of chelation because it cannot complex with additional groups beyond the aldehyde component of the aldol reaction.[11-12]

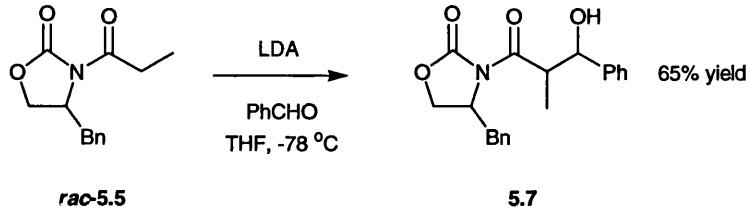
Besides the metal-enolate, the choice of the aldehyde is also crucial. Stereocontrol in the aldol reaction with chiral auxiliaries strongly depends upon the aldehyde structure.[4,5,8,13] Aldehydes such as benzaldehyde and isobutyraldehyde usually provide good diastereocontrol, while aldehydes with small substituents (Me, Et) usually do not.

The following paragraphs describe our studies towards the aldol reaction with a racemic auxiliary. Though the exhaustive literature about stereocontrolled aldol reactions with chiral auxiliaries, no example is reported to date about the use of a racemic auxiliary.
Remember that starting from a racemic 2-oxazolidinone, we wished to obtain only two enantiomeric aldol adducts. If a stereoselective aldol reaction with a chiral auxiliary can afford four possible isomers, the matter is even more complicated when a racemic auxiliary is used and so eight possible isomers can be obtained.
With this main issue to solve, we focused our attention upon the search of the ideal stereoselective reaction. Therefore, we tested different enolates and base systems for this reaction. Whilst, looking for a compromise between a good diastereocontrol in the aldol reaction and a suitable substrate for the enzymatic resolution (Scheme 5.1),[14-16] we also considered the possibility of a less hindered aldehyde such as acetaldehyde.

5.3 Approaching the synthesis of the racemic aldol adduct

5.3.1 Synthesis of aldol adducts *via* Li-enolate and their enzymatic resolution

In the initial screening of reaction diastereoselectivity we decided to use a Li-enolate. The chosen oxazolidinone was the synthetically useful racemic 4-benzyl-2-oxazolidinone auxiliary.[17]
The reaction studied was the aldol reaction of racemic 3-propionyl-4-benzyl-2-oxazolidinone **5.5** with benzaldehyde using LDA as base, at -78 °C in tetrahydrofuran solution (Scheme 5.11).[18]

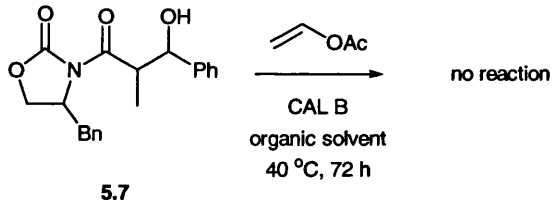


rac-5.5 5.7

Scheme 5.11. The synthesis of aldol adducts *via* Li-enolate

Though a reasonable yield was obtained, the reaction stereoselectivity was very poor, giving different and uncharacterised diastereomers and enantiomers, according to TLC, HPLC and NMR analysis.

To examine the enzymatic activity towards any obtained aldol product, the crude aldol adducts **5.7** were stirred with CAL B under standard conditions,[19] using vinyl acetate as acylating agent in either *n*-hexane or in vinyl acetate (Scheme 5.12).



5.7

Scheme 5.12. The enzymatic resolution of Li-enolate aldol adducts

After 3 days, no reaction or kinetic resolution of the aldol adducts from benzaldehyde was detected by chromatographic and spectroscopic analyses.

The inadequacy of the Li-mediated aldol reaction was obvious. Because of the poor diastereoselectivity, we began to think about a more diastereoselective reaction to use, changing the metal of the enolate complex.

Since no enzymatic reaction was detected, we also considered the idea of a less hindered aldehyde substrate.

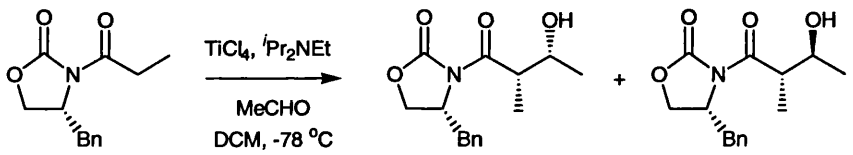
5.3.2 Synthesis of aldol adducts *via* $TiCl_4$/$^iPr_2NEt$-enolate

As a consequence of the poor diastereoselectivity of the Li-enolate mediated aldol reaction, our second attempt focused upon titanium enolates. Given the high

diastereomeric excess obtained in aldol reactions with $TiCl_4$[7] and $ClTi(O^iPr)_3$[8], we decided to investigate the use of Ti-enolates, particularly a tetrachlorotitanium-enolate generated by diisopropylethylamine.

Instead of using the racemic auxiliary directly, as we had done in the case of the Li-enolate mediated aldol reaction, we simplified the matter. The stereochemical outcome of the aldol reaction was determined by reacting (*R*)-3-propionyl-4-benzyl-2-oxazolidinone **(*R*)-5.5** and (*S*)-3-propionyl-4-benzyl-2-oxazolidinone **(*S*)-5.5** separately. Considering the effect of lipase specificity towards the steric bulk of the substituents,[14-16] acetaldehyde was selected instead of benzaldehyde.

First, we examined the aldol reaction between oxazolidinone **(*R*)-5.5** and acetaldehyde in the presence of titaniumtetrachloride and diisopropylethylamine (Scheme 5.13).



(*R*)-5.5 **(4*R*,2'*S*,3'*R*)-5.8** "non-Evans" *syn* **(4*R*,2'*S*,3'*S*)-5.8** *anti 1*

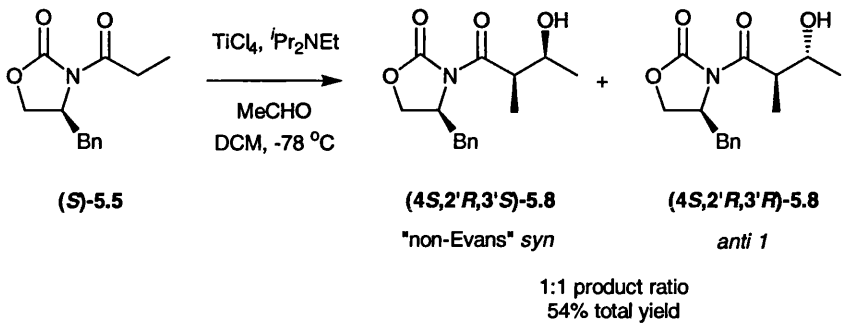
1:1 product ratio
50% total yield

Scheme 5.13. The synthesis of the (4*R*)-aldol adducts *via* $TiCl_4/^iPr_2NEt$-enolate

Of the four possible isomers that could be obtained, we got only the "non-Evans" *syn* and the *anti 1* aldol adducts, in a 1:1 ratio.

The "non-Evans" *syn* and the *anti* relative configurations for aldol adducts **5.8** were established from the observed ^1H NMR coupling constants for the vicinal protons at the newly created stereogenic centres,[8,20,21] by comparison with analogous compounds synthesised by us in subsequent studies and by enzymatic resolution of the diastereomeric product (see below).

Given that the reaction with the (R)- enantiomer proceeded diastereoselectively, we wanted then to ensure that the same diastereoselectivity could be observed when the other enantiomer was used. Oxazolidinone **(*S*)-5.5** was reacted in the same reaction conditions, as shown in Scheme 5.14.



Scheme 5.14. The synthesis of the (4*S*)-aldol adducts *via* $TiCl_4$/$^iPr_2NEt$-enolate

Again, only the "non-Evans" *syn* and the *anti 1* aldol adducts were formed in 1:1 ratio.

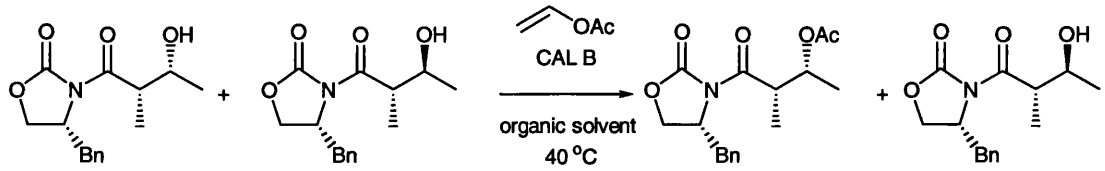
In summary, each enantiomer of the acylated oxazolidinone **5.5** gave as the major products two of the four possible diastereomers of aldol adduct **5.8**, according to chiral HPLC and NMR analysis. The given yield is the total yield of the aldol mixture, calculated after chromatographic purification.

5.3.3 Enzymatic kinetic resolution of the diastereomeric aldol adducts from $TiCl_4$/$^iPr_2NEt$-enolate mediated aldol reaction

Each pair of aldol adducts was tested in the enzymatic resolution. Lipase from *Candida antarctica* type B was again used as catalyst for its general high activity, selectivity and versatility in transesterification reactions in organic solvent.[22-25]

5.3.3.1 Enzymatic resolution of aldols (4*R*,2'*S*,3'*R*)- and (4*R*,2'*S*,3'*S*)-5.8

The enzymatic resolution of aldols **(4*R*,2'*S*,3'*R*)-** and **(4*R*,2'*S*,3'*S*)-5.8** from oxazolidinone **(*R*)-5.5** was carried out using vinyl acetate in different organic solvents at 40 °C, catalysed by lipase from *Candida antarctica* type B (Scheme 5.15).



(4*R*,2'*S*,3'*R*)-5.8 (4*R*,2'*S*,3'*S*)-5.8 (4*R*,2'*S*,3'*R*)-5.9 "non-Evans" *syn* (4*R*,2'*S*,3'*S*)-5.8 *anti 1*

Scheme 5.15. The enzymatic resolution of aldol adducts

The enzymatic resolution was successful. The (3'*R*) diastereomer reacted faster than the (3'*S*) one.

The progress and the diastereomeric excess of the reaction were monitored by chiral HPLC analysis after removal of the enzyme by filtration.

In Table 5.1, the transesterification rate and diastereoselectivity of the enzymatic reaction in vinyl acetate are illustrated.

Table 5.1. Time course and diastereoselectivity of the enzymatic reaction in vinyl acetate[a] illustrated in Scheme 5.15

t(h)	%conv.	%d.e.$_p$[b]	%d.e.$_s$[c]	D[d]
0	0	-	0	-
1	0	-	0	-
4	6	>99	6	>210
24	34	>99	51	>330
48	43	>99	75	>440

[a] 50 $mg_{enzyme}/mmol_{substrate}$ in 5 mL vinyl acetate using 1 mmol of diastereomeric aldo.l

[b] diastereomeric excess of the product.

[c] diastereomeric excess of the remaining substrate.

[d] $D = \ln[(1-c(1+d.e._p)]/\ln[(1-c(1-d.e._p)] = \ln[(1-c)(1-d.e._s)]/\ln[(1-c)(1+d.e._s)]$; c= conversion. [26,27]

The extent of conversion of the diastereomeric starting material and the optical purity expressed as the diastereomeric excess was calculated according to the equation previously described in literature.[26,27] The diastereomeric ratio D was calculated from the extent of conversion and the d.e. of the product according to the equation generally used to calculate E (enantiomeric ratio).[27]

Table 5.1 shows that the conversion increased up to 43% after 48 h when vinyl acetate was used as both solvent and acylating agent. Only one diastereomer was acylated during the course of the reaction; this explains why a diastereomeric excess >99% for the product was always observed. On the other hand, the diastereomeric excess of the substrate increased with time from 0 up to 75%: the percentage of the slower reacting aldol adduct increased as the other one was acylated to the corresponding acyl ester **5.9**.

The relationship between the conversion and the diastereomeric excess of the product (or the remaining substrate) is expressed by D, the diastereomeric ratio. D gives a value of the discrimination of two competing diastereomers by the enzyme.[26,27] In our case, the diastereoselectivity of the reaction, expressed as D, increased with reaction time: the enzyme preferred the (3'*R*) diastereomer as substrate, even when its percentage in the diastereomeric substrate [(3'*R*) + (3'*S*)] decreased.

The same experiment was repeated in solvents with different hydrophobicity, as illustrated in Table 5.2.

Table 5.2. Time course and diastereoselectivity of the enzymatic reaction illustrated in Scheme 5.15 in different solvents[a]

DCM					*n*-Hexane				
t(h)	%conv.	%d.e.$_p$[b]	%d.e.$_s$[c]	D[d]	t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D
0	0	-	0	-	0	0	-	0	-
1	0	-	0	-	3	30	>99	42	>300
4	2	>99	2	>200	6	46	>99	84	>530
24	6	>99	6	>210	24	50	>99	>99	>1050
48	12	>99	13	>220	48	56	88	>99	-

Toluene					Diisopropyl ether				
t(h)	%conv.	%d.e.$_p$	%d.e.$_s$	D	t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D
0	0	-	0	-	0	0	-	0	-
1	0	-	0	-	1	0	-	0	-
4	12	>99	13	>220	4	16	>99	19	>230
24	42	>99	71	>420	24	45	>99	81	>500
48	45	>99	81	>500	48	45	>99	81	>500

[a] 50 $mg_{enzyme}/mmol_{substrate}$ in 5 mL solvent using 1 mmol of diastereomeric aldol and 2 mmol vinyl acetate.

[b] diastereomeric excess of the product.

[c] diastereomeric excess of the remaining substrate.

[d] $D = \ln[(1-c(1+d.e._p)]/\ln[(1-c(1-d.e._p)] = \ln[(1-c)(1-d.e._s)]/\ln[(1-c)(1+d.e._s)]$; c= conversion.[26,27]

The kinetics of the reactions in different solvents was reported in graphs.
Fig. 5.1 displays the conversion (%) versus the reaction time.

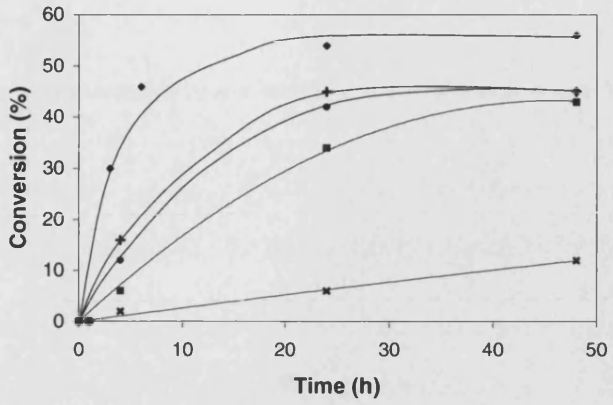


Fig. 5.1. Rate of transesterification in different solvents: ◆ *n*-hexane, • toluene, + isopropyl ether, ▪ vinyl acetate, × DCM

The reaction rate of the lipase-catalysed transesterification was faster in non-polar solvents such as *n*-hexane and toluene, and slower in polar solvents such as DCM.[28-30]

The $d.e._p$ values reported in Table 5.2 show that after 48 hours, only one of the two diastereomers was converted into the corresponding ester **(4*R*,2'*S*,3'*R*)-5.9** in vinyl acetate, DCM, isopropyl ether and toluene (d.e. >99%). The other ester **(4*R*,2'*S*,3'*S*)-5.9** appeared only in *n*-hexane after 24 hours and the diastereomeric excess of the product decreased as the reaction progressed.

In Fig. 5.2, the diastereomeric excess of the substrate in the different solvents is plotted against the reaction time.

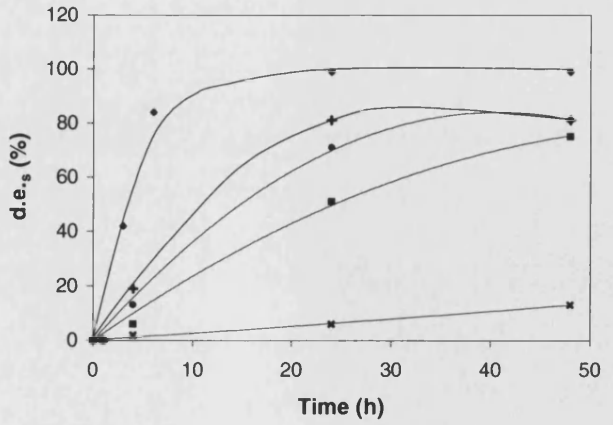


Fig. 5.2. Time course of the d.e. of the substrate in different solvents: ◆ *n*-hexane, • toluene, + isopropyl ether, ▪ vinyl acetate, × DCM

The increase in diastereomeric excess of the substrate (Fig. 5.2) was faster in *n*-hexane and considerably slower in DCM. The results achieved in toluene, diisopropyl ether and vinyl acetate solution were comparable.

A common trend could be detected when the d.e. of the substrate was plotted against the conversion for each of the different solvents (Fig. 5.3).

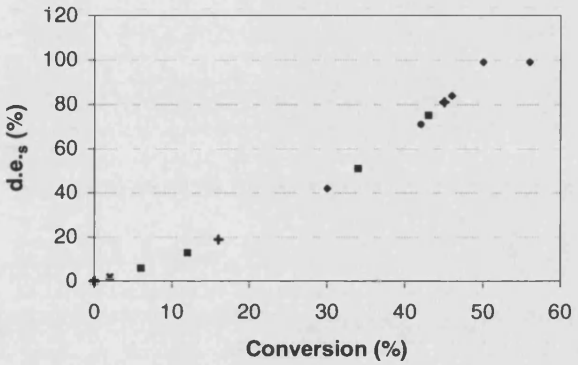


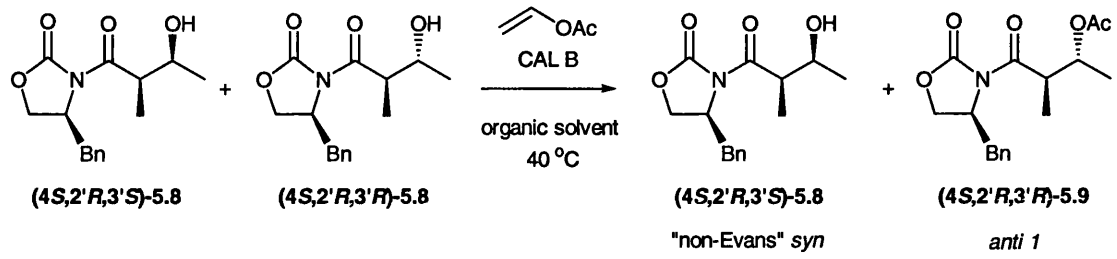
Fig. 5.3. Diastereoselectivity vs. conversion: ◆ *n*-hexane, • toluene, + isopropyl ether, ▪ vinyl acetate, × DCM

As the conversion increased, the d.e. of the remaining substrate increased, because one of the two diastereomers was preferentially acetylated. The other diastereomer reacted only when the preferred one was completely consumed.

5.3.3.2 Enzymatic resolution of aldols (4*S*,2'*R*,3'*S*)- and (4*S*,2'*R*,3'*R*)-5.8

The enzymatic resolution of the two aldol adducts from oxazolidinone **(*R*)-5.5** was a success, with the enzyme preferring the (3'*R*) diastereomer in the acylation reaction. Our next step was to find out if the same diastereoselectivity could be observed when the aldol adducts from oxazolidinone **(*S*)-5.5** were reacted with vinyl acetate in the presence of CAL B.

The enzymatic resolution of aldols **(4*S*,2'*R*,3'*S*)-** and **(4*S*,2'*R*,3'*R*)-5.8** from oxazolidinone **(*S*)-5.5** was carried out as described above for aldols from **(*R*)-5.5** (Scheme 5.16).



Scheme 5.16. The enzymatic resolution of the adducts from $TiCl_4$/$^iPr_2NEt$ aldol reaction

As we expected, the (3'*R*) diastereomer reacted faster than the (3'*S*) diastereomer. Conversion and enantiomeric excess were similar to those obtained when the aldol adducts from **(*R*)-5.5** were enzymatically resolved.

The progress and the diastereomeric excess of the reaction were monitored by chiral HPLC analysis after removal of the enzyme by filtration.

In Table 5.3 the time course and diastereoselectivity of the enzymatic reaction in vinyl acetate are reported.

Table 5.3. Time course and diastereoselectivity of the enzymatic reaction illustrated in Scheme 5.16 in vinyl acetate[a]

t (h)	%conv.	%d.e.$_p$[b]	%d.e.$_s$[c]	D[d]
0	0	-	0	-
1	0	-	0	-
4	3	>99	3	>200
8	9	>99	10	>210
24	29	>99	40	>290
48	47	>99	88	>580

[a] 50 $mg_{enzyme}/mmol_{substrate}$ in 5 mL vinyl acetate using 1 mmol of diastereomeric aldol.

[b] diastereomeric excess of the product.

[c] diastereomeric excess of the remaining substrate.

[d] $D = \ln[(1-c(1+d.e._p)]/\ln[(1-c(1-d.e._p)] = \ln[(1-c)(1-d.e._s)]/\ln[(1-c)(1+d.e._s)]$; c= conversion. [26,27]

The conversion increased up to 47% with reaction time. After 48 h only one diastereomer was acylated (d.e.$_p$ >99%). Whilst the diastereomeric excess of the

substrate increased from 0 up to 88%; the percentage of the slower reaction diastereomer increased as the other one was acylated. Diastereoselectivity (D) of the reaction increased with the reaction time.

The reaction was repeated in a range of solvents with different hydrophobicity (Table 5.4). Traces of the other acylated diastereomer were detected in *n*-hexane, toluene and diisopropyl ether.

Table 5.4. Time course and diastereoselectivity of the enzymatic reaction illustrated in Scheme 5.16 in different solvents[a]

Toluene					Diisopropyl ether				
t (h)	%conv.	%d.e.$_p$[b]	%d.e.$_s$[c]	D[d]	t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D
0	0	-	0	-	0	0	-	0	-
2	0	-	0	-	2	5	>99	5	>200
4	3	>99	3	>200	4	7	>99	7	>210
6	5	>99	5	>200	6	11	>99	12	>220
24	28	90	35	>20	24	49	92	88	>70
48	44	88	69	>30	48	56	88	>99	-

DCM					Dioxane				
t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D	t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D
0	0	0	-	-	0	0	-	0	-
1	0	0	-	-	2	0	-	0	-
4	0	0	-	-	4	0	-	0	-
8	3	>99	3	>200	6	0	-	0	-
24	7	>99	7	>210	24	5	>99	5	>200
48	21	>99	26	>250	48	8	>99	9	>216

n-Hexane				
t (h)	%conv.	%d.e.$_p$	%d.e.$_s$	D
0	0	-	0	-
1	3	>99	3	>200
4	18	>99	22	>240
8	39	>99	63	>380
24	51	89	>99	>50
48	60	80	>99	-

[a] 50 $mg_{enzyme}/mmol_{substrate}$ in 5 mL solvent using 1 mmol of diastereomeric aldol and 2 mmol vinyl acetate.

[b] diastereomeric excess of the product.

[c] diastereomeric excess of the remaining substrate.

[d] $D = \ln[(1-c(1+d.e._p)]/\ln[(1-c(1-d.e._p)] = \ln[(1-c)(1-d.e._s)]/\ln[(1-c)(1+d.e._s)]$; c= conversion.[26,27]

We studied the kinetics of the reactions performed in the different solvents.
In Fig. 5.4 the conversion (%) is displayed against the reaction time. Again the observed reaction rate was faster in less polar solvent.

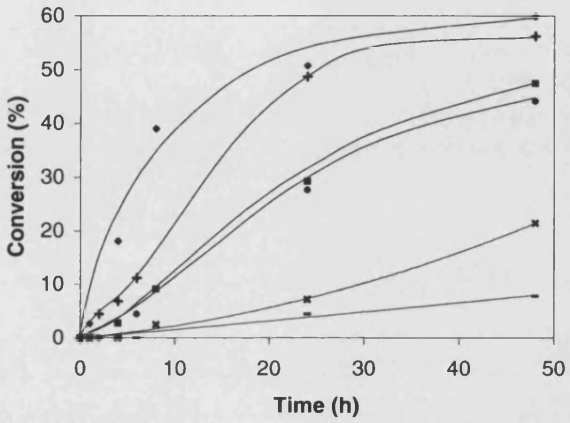


Fig. 5.4. Rate of transesterification in different solvent: ◆ *n*-hexane, • toluene, + isopropyl ether, ▪ vinyl acetate, × DCM, - dioxane

In Fig. 5.5 and Fig. 5.6 the diastereomeric excess of the product and the diastereomeric excess of the substrate in the different solvents are respectively plotted versus the reaction time.

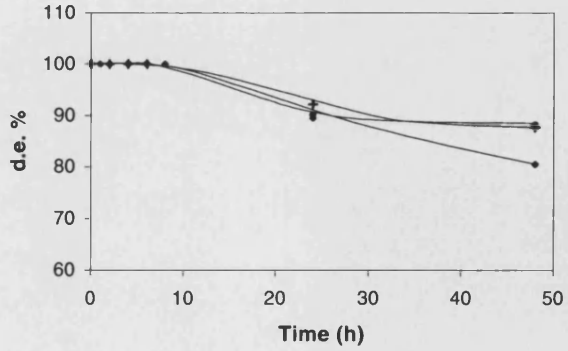


Fig. 5.5. Time course of d.e. of the product in different solvents: ◆ *n*-hexane, • toluene, + isopropyl ether

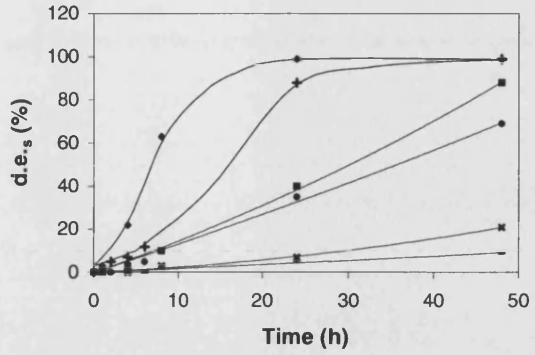


Fig. 5.6. Time course of d.e. of the substrate in different solvents: ◆ *n*-hexane, • toluene, + isopropyl ether, ▪ vinyl acetate, × DCM, - dioxane

After 48 hours, only aldol adduct **(4*S*,2'*R*,3'*R*)-5.8** was converted into the corresponding acetate in vinyl acetate, DCM and dioxane. The acetate from aldol **(4*S*,2'*R*,3'*S*)-5.8** appeared in *n*-hexane, toluene and isopropyl ether after 24 hours and the diastereomeric excess of the product in these solvents decreased as the reaction progressed (Fig. 5.5).

The increase in diastereomeric excess of the substrate (Fig. 5.6) was observed to be faster in *n*-hexane and slower in the other solvents screened.

In Fig. 5.7 the diastereomeric excess of the substrate is reported against the conversion for the solvents examined.

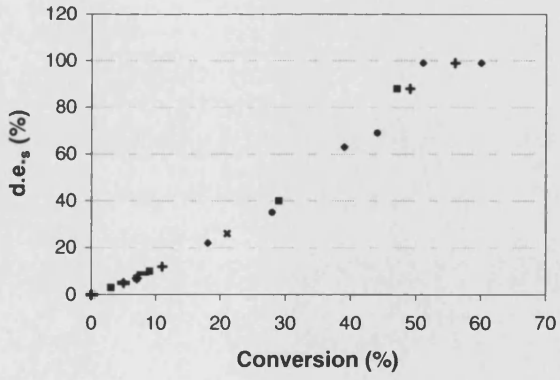
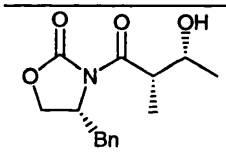
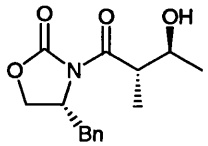
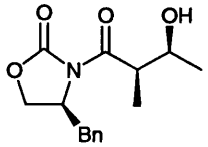
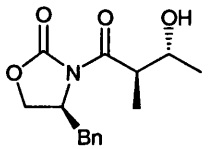


Fig. 5.7. Diastereoselectivity vs. conversion

Again a common trend can be noted. One of the two diastereomers reacts selectively, and the other starts to react only when the first one has been completely consumed.

We had found a diastereoselective aldol reaction, which employed $TiCl_4/^iPr_2NEt$-enolate. Only two of the four possible diastereomers were formed from each enantiomer of the acylated oxazolidinone **5.5** in a 1:1 mixture.
The use of CAL B as catalyst was found to be a valid method for the resolution of diastereomers of aldol adducts from acetaldehyde. The reaction was relatively fast when *n*-hexane was used as solvent (> 40% conversion in 8 h) and proceeded with high diastereoselectivity (>99% d.e.). Moreover, the (3'*R*) diastereomers were preferentially acylated by the enzyme, as reported in Table 5.5.

Table 5.5. Chiral preference of CAL B in the acylation reaction

Aldol 5.8	Configuration	Enzyme substrate
	(4*R*,2'*S*,3'*R*)	✓
	(4*R*,2'*S*,3'*S*)	no
	(4*S*,2'*R*,3'*S*)	no
	(4*S*,2'*R*,3'*R*)	✓

These results were very promising. We had demonstrated the validity of our approach of coupling a diastereoselective aldol reaction to an enzymatic resolution. We wanted a diastereoselective aldol reaction and we found one able to give a 1:1

mixture. We wanted to see if enzymes could react selectively with only one diastereomer. They did.

We would expect that by means of an aldol reaction with better stereoselectivity, we might be able to obtain only one diastereomer from each enantiomer of *N*-propionyl oxazolidinone **5.5**, *i.e.* two enantiomers, separable upon the chiral preference of the lipase towards (3'*R*) stereocentres.

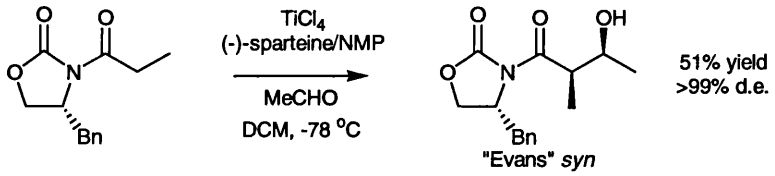
5.3.4 Synthesis of aldol adducts *via* $TiCl_4$/(-)-sparteine/NMP-enolate and their enzymatic resolution

We still needed to improve the stereoselectivity of the aldol reaction with acetaldehyde. Our aim was always to obtain only one isomer from each enantiomer of oxazolidinones **(*R*)-5.5** and **(*S*)-5.5**, in order to get closer to the use of racemic auxiliaries (Scheme 5.1).

The desired stereoselectivity for the aldol reaction was obtained when we used the same reaction conditions reported by Crimmins and co-workers for an analogous asymmetric aldol reaction.[31]

The acylated oxazolidinones **(*R*)-5.5** and **(*S*)-5.5** were separately reacted with acetaldehyde *via* a $TiCl_4$-enolate using a combination of (-)-sparteine and *N*-methyl-2-pyrrolidinone (1:1) as base.

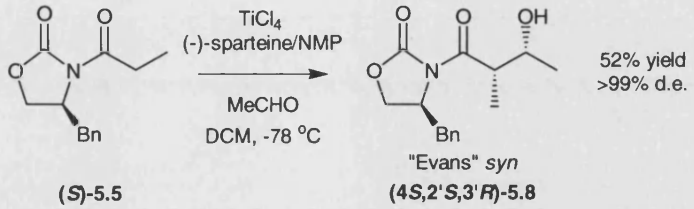
The acylated oxazolidinones **(*R*)-5.5** afforded the "Evans" *syn* aldol adduct **5.8** in reasonable yield and >99% diastereomeric excess (Scheme 5.17).



(*R*)-5.5 **(4*R*,2'*R*,3'*S*)-5.8**

Scheme 5.17. Synthesis of aldol adducts *via* $TiCl_4$ /(-)-sparteine/NMP-enolate from acylated oxazolidinone **(*R*)-5.5**

Using the same reaction, the acylated oxazolidinones **(*S*)-5.5** also afforded the "Evans" *syn* aldol adduct **5.8** in reasonable yield and >99% diastereomeric excess (Scheme 5.18).

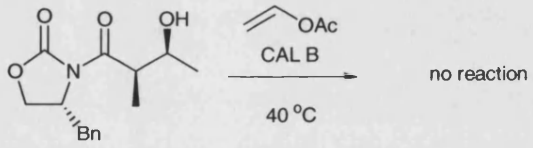


(*S*)-5.5 **(4*S*,2'*S*,3'*R*)-5.8**

Scheme 5.18. Synthesis of aldol adducts *via* $TiCl_4$/(-)-sparteine/NMP-enolate from acylated oxazolidinone **(*S*)-5.5**

Each enantiomer gave only the "Evans" *syn* aldol in 99% diastereomeric excess, according to chiral HPLC analysis. The "Evans" *syn* relative configurations of aldols **(4*R*,2'*R*,3'*S*)-5.8** and **(4*S*,2'*S*,3'*R*)-5.8** were established by comparison of the specific rotations and ^{1}H NMR spectra with those reported in the literature.[32]

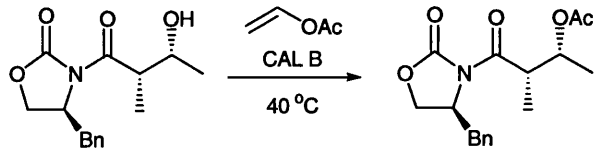
After purification by chomatography, the enantiomers were examined for their reactivity towards acylation, using *Candida antarctica* type B as the catalyst.
"Evans" *syn* aldol adduct **(3'*S*)-5.8** was not acylated by the enzyme (Scheme 5.19).



(4*R*,2'*R*,3'*S*)-5.8
"Evans" *syn*

Scheme 5.19. Enzymatic resolution of "Evans" *syn* aldol adduct **(3'*S*)-5.8** from the $TiCl_4$/$^i Pr_2NEt$-enolate aldol reaction

"Evans" *syn* aldol adduct **(3'*R*)-5.8** was successfully acylated, as illustrated in Scheme 5.20.



(4*S*,2'*S*,3'*R*)-5.8
"Evans" *syn*

(4*S*,2'*S*,3'*R*)-5.9

Scheme 5.20. Enzymatic resolution of "Evans" *syn* aldol adduct **(3'*R*)-5.8** from the $TiCl_4$/$^iPr_2NEt$-enolate aldol reaction

No enzymatic reaction of aldol **(4*R*,2'*R*,3'*S*)-5.8** was detected by TLC and chiral HPLC analysis. However, aldol adduct **(4*S*,2'*S*,3'*R*)-5.8** was converted into ester **(4*S*,2'*S*,3'*R*)-5.9** in 87% conversion after 72 hours.
The time course of the enzymatic reaction is illustrated in Table 5.6 and Fig. 5.8.

Table 5.6. Time course of the enzymatic reaction illustrated in Scheme 5.20[a]

Time (h)	Conversion (%)
0	0
1.5	2
4	8
8.5	15
24	30
48	47
72	87

[a] 30 $mg_{enzyme}/mmol_{substrate}$ in 5 mL vinyl acetate using 1 mmol of aldol **(4*S*,2'*S*,3'*R*)-5.8**.

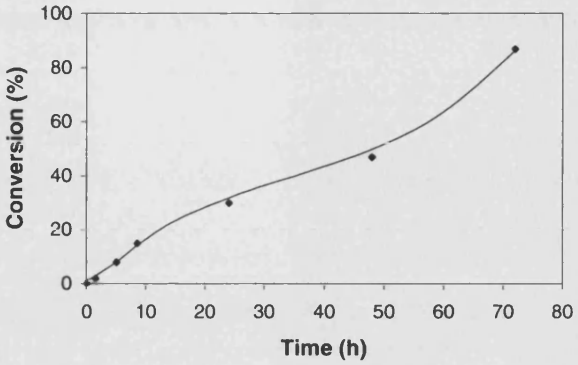
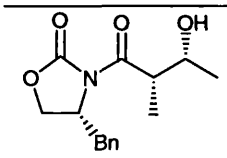
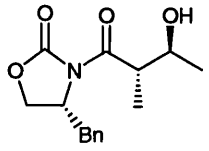
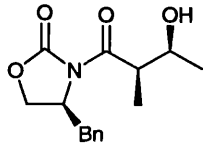
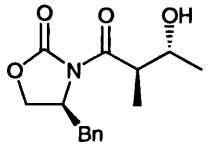
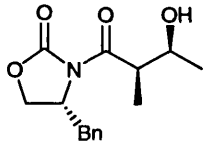
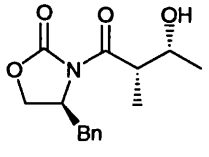


Fig. 5.8. Rate of transesterification

These results were compared with those previously obtained in the lipase-catalysed resolution of analogous isomers of aldol adduct **5.8** (Table 5.7).

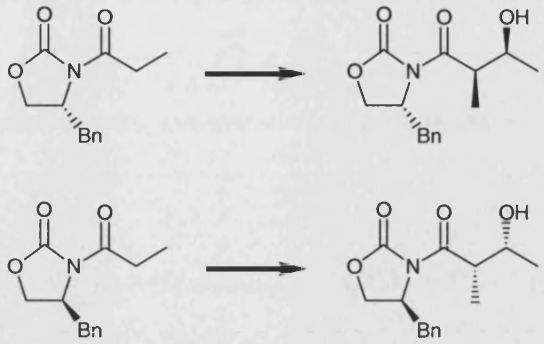
Table 5.7. Chiral preference of CAL B in the acylation reaction

Aldol 5.8	Configuration	Enzyme substrate
	(4*R*,2'*S*,3'*R*)	✓
	(4*R*,2'*S*,3'*S*)	no
	(4*S*,2'*R*,3'*S*)	no
	(4*S*,2'*R*,3'*R*)	✓
	(4*R*,2'*R*,3'*S*)	no
	(4*S*,2'*S*,3'*R*)	✓

From these results, the chiral preference of *Candida antarctica* lipase B towards (3'*R*)-stereocentres in the acetylation reaction was obvious.[16,33]

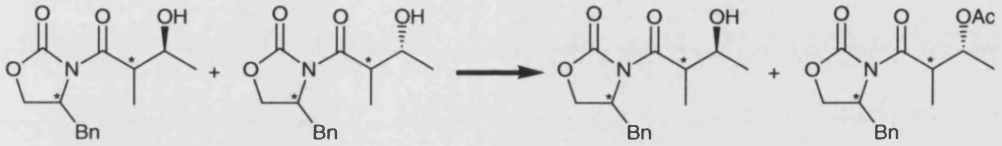
5.4 Synthesis of the racemic aldol adduct and its enzymatic resolution

At this point of our investigation we had two powerful tools to work with:
A) We had found a highly stereoselective and reliable aldol reaction able to afford only one diastereomer from each enantiomer of the acylated chiral auxiliary. These aldols were therefore also enantiomeric, as illustrated in Scheme 5.21:



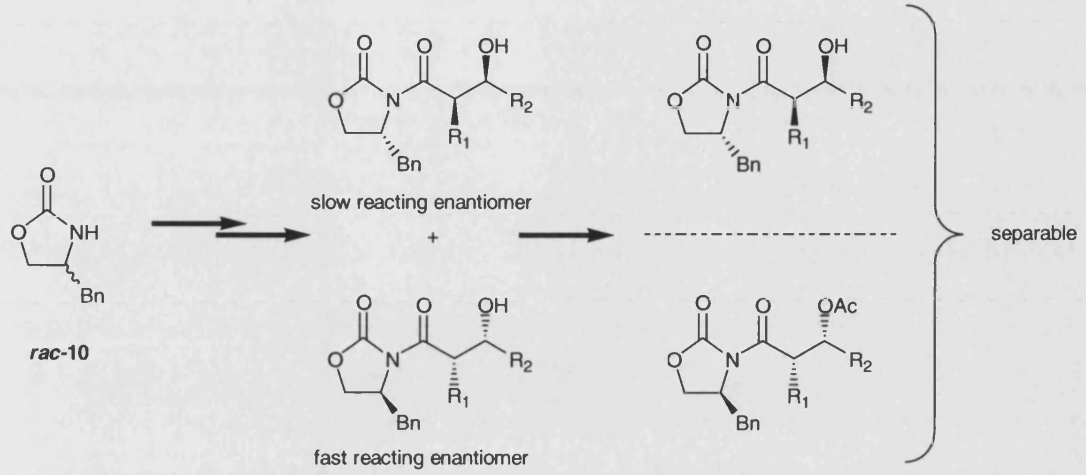
Scheme 5.21

B) We had established that CAL B promoted the kinetic resolution of our aldol adducts, preferring the (*R*)- to the (*S*)-stereocentre at the carbinol carbon (Scheme 5.22):



Scheme 5.22

Research next focused upon the combination of these results, using a racemic starting material. We wanted to see if the highly diastereoselective aldol reaction *via* $TiCl_4$/(-)-sparteine/NMP-enolate was efficient when a racemic mixture of oxazolidinone **5.10** was used instead of the two individual enantiomers. We wished to obtain a racemic mixture of diastereomerically pure aldol adducts, where one enantiomer would undergo enzyme-catalysed acylation faster (Scheme 5.23).



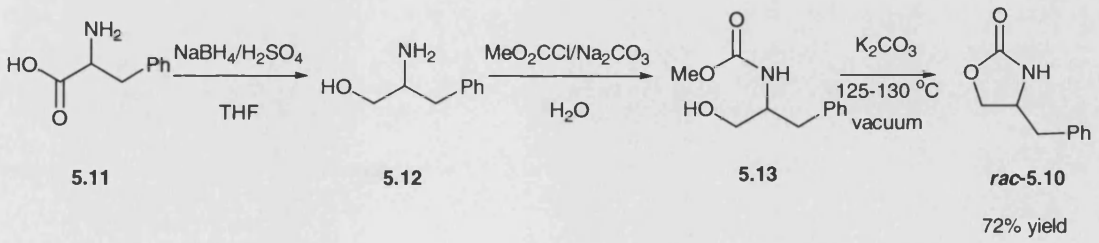
Scheme 5.23

Successful completion of the reaction illustrated in this scheme would equate to successful completion of the project's initial aims.

5.4.1 Synthesis of racemic *N*-(3-acetoxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone and its enzymatic resolution

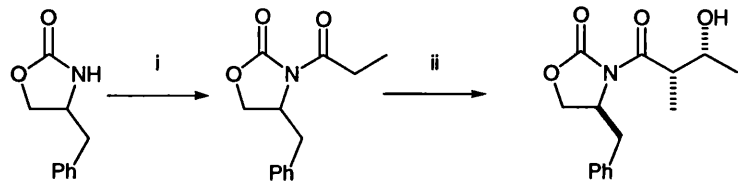
Racemic 4-benzyl-2-oxazolidinone **5.10** was prepared from the corresponding racemic amino acid phenylalanine **5.11**, following the procedure reported by Wu and Shen.[34]

The racemic amino alcohol **5.12** (from reduction of amino acid **5.11** with $NaBH_4/H_2SO_4$[35]) was treated directly with MeO_2CCl to give the carbamate **5.13**, which was cyclised in the presence of traces of K_2CO_3 at 125-130 °C under vacuum, to afford the racemic 2-oxazolidinone **5.10** in 72% total yield (Scheme 5.24).



Scheme 5.24. Synthesis of the racemic 4-benzyl-2-oxazolidinone **5.10**

The racemic oxazolidinone **5.10** was then acylated to obtain the racemic 3-propionyl-4- benzyl-2-oxazolidinone **5.5**.[36] The subsequent diastereoselective aldol reaction with acetaldehyde in the presence of $TiCl_4$, (-)-sparteine and *N*-methyl-2-pyrrolidinone[31] provided racemic ***syn*-5.8** in 99% diastereomeric excess (Scheme 5.25).



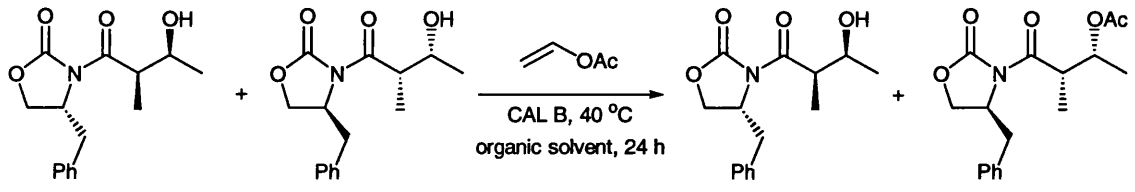
rac-5.10 *rac*-5.5 "Evans" *rac-syn*-5.8

(i) ^{n}BuLi, EtCOCl/THF, -78 °C (88%); (ii) $TiCl_4$, (-)-sparteine, *N*-methyl-2-pyrrolidinone, CH_3CHO/DCM, 0 → -78 °C (54%)

Scheme 5.25. Acylation and aldol reaction of *rac*-4-benzyl-2-oxazolidinone **5.10**

Sparteine was used as a convenient base and did not, as expected, exert any observable stereocontrol upon the aldol reaction.

Enzymatic resolution of the enantiomers of aldol adduct **5.8** *via* transesterification catalysed by *Candida antarctica* lipase type B, afforded the (3'*R*)-acylated aldol adduct **5.9** and the (3'*S*)-non-acylated aldol adduct **5.8** (Scheme 5.26). The choice of solvent was crucial to this process, and the solvent effects are illustrated in Table 5.8.



(4*R*,2'*R*,3'*S*)-5.8 (4*S*,2'*S*,3'*R*)-5.8 (4*R*,2'*R*,3'*S*)-5.8 (4*S*,2'*S*,3'*R*)-5.9

Scheme 5.26. Lipase-catalysed aldol adduct resolution

Table 5.8. Influence of solvent on conversion and enantiomeric excess[a]

Entry	Solvent	Conversion %	(4*R*,2'*R*,3'*S*)-5.8 e.e. %	(4*S*,2'*S*,3'*R*)-5.9 e.e. %	E[b]
1	*n*-hexane	50	>99	>99	>1050
2	$^{i}Pr_2O$	45	81	>99	>500
3	toluene	42	72	>99	>420
4	VA	34	51	>99	>330
5	DCM	6	6	>99	>210

[a] 30 $mg_{enzyme}/mmol_{substrate}$ in 5 mL solvent using 1 mmol of racemic aldol and 2 mmol vinyl acetate.

[b] $E = \ln[(1-c(1+e.e._p)]/\ln[(1-c(1-e.e._p)] = \ln[(1-c)(1-e.e._s)]/\ln[(1-c)(1+e.e._s)]$; $e.e._s$= enantiomeric excess of the remaining substrate; $e.e._p$= enantiomeric excess of the product; c = conversion.[26,27]

The reaction rate of the lipase-catalysed transesterification was faster in non-polar solvents such as *n*-hexane and toluene (Entries 1 and 3), whilst it was slower in polar solvents such as dichloromethane (Entry 5).[28-30]

The selectivity of *Candida antarctica* lipase type B towards the aldol adduct enantiomers was in accordance with the reported stereoselectivity of this enzyme towards racemic secondary alcohols.[37]

The enantiomeric excess of the recovered **5.8** and of product **5.9** was monitored during the course of the reaction in *n*-hexane solution (Fig. 5.9). The enantiomeric excess of the product was constant and >99% during the reaction. The enantiomeric excess of the substrate increased to 99% as the reaction approached 50% conversion.

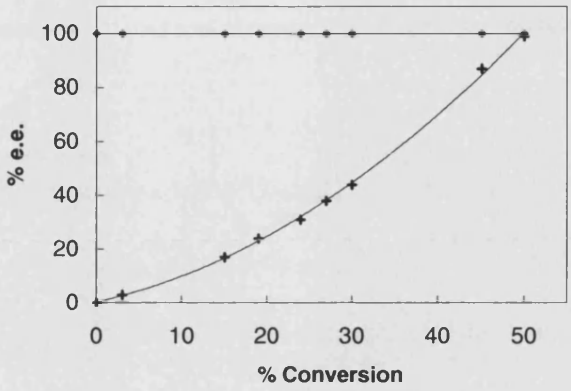
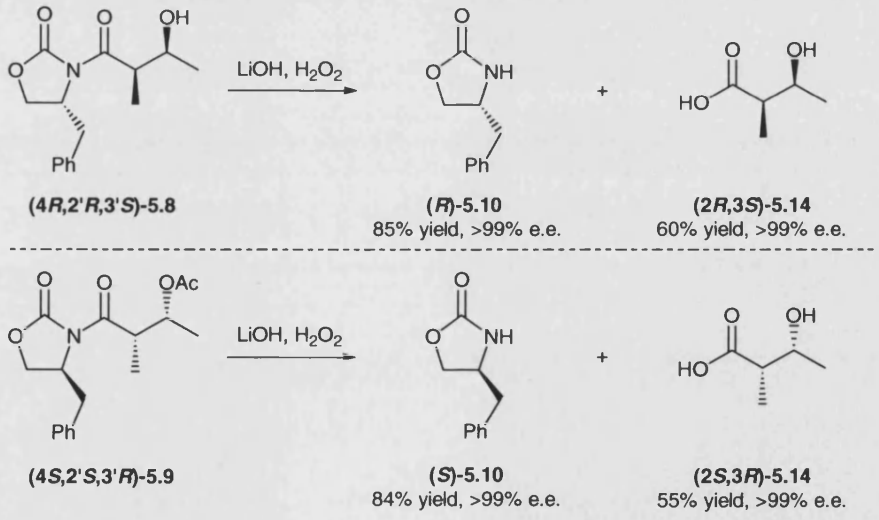


Fig. 5.9. Kinetic study of enantiomeric excess versus conversion in *n*-hexane: ♦acetylated aldol adduct, + non-acetylated aldol adduct

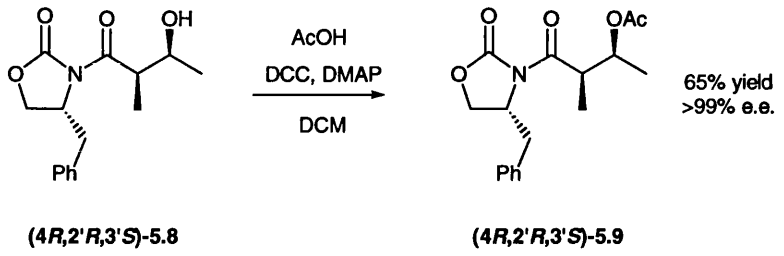
Finally, hydrolysis[36] of the enzymatically resolved aldol **5.8** and ester **5.9** afforded the enantiomerically enriched 3-hydroxy-2-methylbutanoic acids **(2*R*,3*S*)-5.10** and **(2*S*,3*R*)-5.10** and the enantiomerically enriched 2-oxazolidinones **(*R*)-5.14** and **(*S*)-5.14** (Scheme 5.27).



Scheme 5.27. Hydrolysis of enzymatic resolution products

The relative configuration of the non-acylated aldol adduct was also confirmed by X-ray crystallographic analysis of the crystal structure (see Appendices).

For comparison, the enantiomer of the lipase-acylated oxazolidinone **5.9** was synthesised by chemical esterification of **(4*R*,2'*R*,3'*S*)-5.8** with acetic acid in the presence of DCC and dimethylaminopyridine (Scheme 5.28).[38]



(4*R*,2'*R*,3'*S*)-5.8 **(4*R*,2'*R*,3'*S*)-5.9**

Scheme 5.28. Chemical esterification of the **(3'*S*)**-aldol

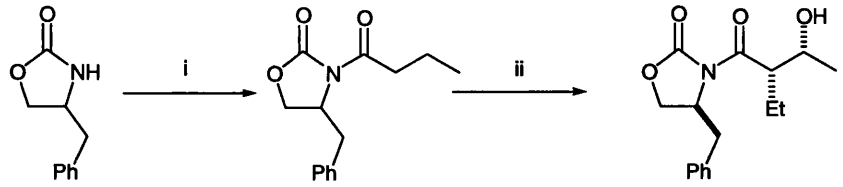
The chemical esterification gave, as expected, the ester with opposite specific rotation with respect to the ester obtained by the enzymatic reaction.

5.4.2 Synthesis of racemic *N*-(3-acetoxy-2-ethylbutanoyl)-4-benzyl-2-oxazolidinone and its enzymatic resolution

To prove the general application of our methodology to obtain enantiomerically enriched auxiliary and enantiomerically enriched product, we decided to change the acyl moiety of our oxazolidinone.

We intended to see if this structural change could affect the aldol reaction diastereoselectivity or/and the enzymatic resolution.

This time, the racemic oxazolidinone **5.10** was acylated using butyryl chloride to obtain the *N*-butanoyl oxazolidinone **5.15**. This underwent the diastereoselective aldol reaction with acetaldehyde in the presence of $TiCl_4$/(-)-sparteine/NMP to afford the ***rac-syn*-5.16** in 59% yield and 95% d.e., according to ^1H NMR[20,32] and chiral HPLC analysis (Scheme 5.29).

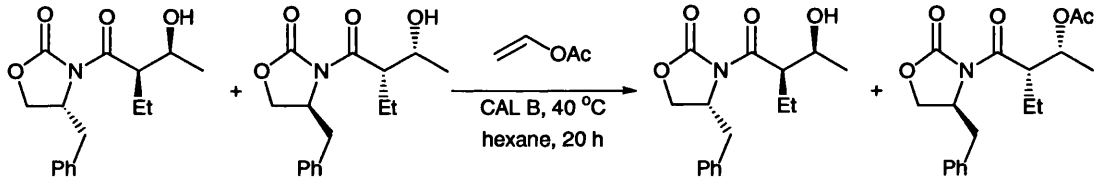


rac-5.10 *rac*-5.15 "Evans" *rac-syn*-5.16

(i) ^{n}BuLi, ^{n}PrCOCl/THF, -78 °C (88%); (ii) $TiCl_4$, (-)-sparteine, *N*-methyl-2-pyrrolidinone, CH_3CHO/DCM, 0 → -78 °C (59%)

Scheme 5.29. Acylation of (±)-4-benzyl-2-oxazolidinone **5.10** with ^{n}PrCOCl and subsequent aldol reaction

Enzymatic resolution of the enantiomers of aldol adduct **5.16** *via* transesterification catalysed by *Candida antarctica* lipase type B in *n*-hexane, afforded the acylated aldol adduct **5.17** and the non-acylated aldol adduct **5.16**[32] with 50% conversion and >99% enantiomeric excess of the product after 20 hours (Scheme 5.30).



(4*R*,2'*R*,3'*S*)-5.16 (4*S*,2'*S*,3'*R*)-5.16 (4*R*,2'*R*,3'*S*)-5.16 >99% e.e. (4*S*,2'*S*,3'*R*)-5.17 50% conv., >99% e.e.

Scheme 5.30. Lipase-catalysed aldol adduct resolution

X-ray analysis of the crystals confirmed the configuration of the non-acylated product **5.16**.

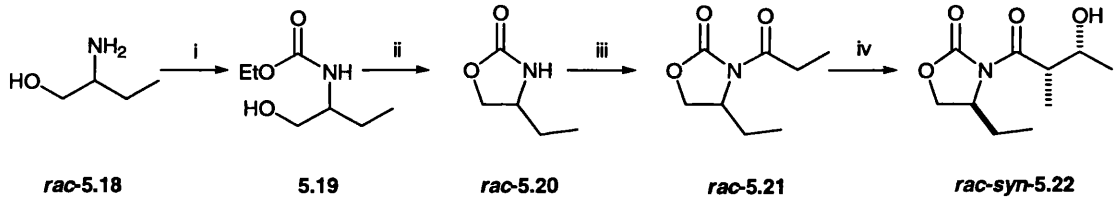
We were very pleased to discover that the aldol reaction was still highly selective even when the acyl moiety was changed. The enzymatic resolution was effective even when the 2'-methyl substituent was exchanged with an ethyl group.

5.5 A general application. Preparation of enantiomerically enriched 4-ethyl-2-oxazolidinones

Our methodology proved to be very efficient when the widely used racemic 4-benzyl-2-oxazolidinone was used as starting material. We were able to obtain enantiomerically enriched β-hydroxy acids and, at the same time, enantiomerically enriched 4-benzyl-2-oxazolidinones. Our aim was now to prove that our approach could be generally exploited to obtain the indirect resolution of chemically designed racemic auxiliaries.

For example, racemic 2-amino-1-butanol is readily available at low cost. We envisaged that using this inexpensive starting material we could obtain enantiomerically enriched 4-ethyl-2-oxazolidinones.

Racemic 2-amino-1-butanol **5.18** was used to synthesise racemic ***rac-syn*-5.22**, following the procedure above described for the synthesis of ***rac-syn*-5.8** (Scheme 5.31).

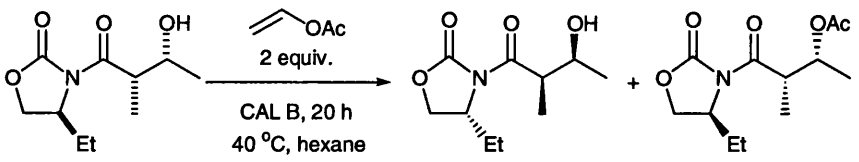


***rac*-5.18** **5.19** ***rac*-5.20** ***rac*-5.21** ***rac-syn*-5.22**

(i) Na_2CO_3/EtO_2CCl, H_2O; (ii) K_2CO_3, 125-130 °C, vacuum (90%); (iii) ^nBuLi, EtCOCl/THF, -78 °C (64%); (iv) $TiCl_4$, (-)-sparteine, *N*-methyl-2-pyrrolidinone, CH_3CHO/DCM, 0 → -78 °C (55%)

Scheme 5.31. The synthesis of the racemic ***rac-syn*-5.22** 4-ethyl oxazolidinone

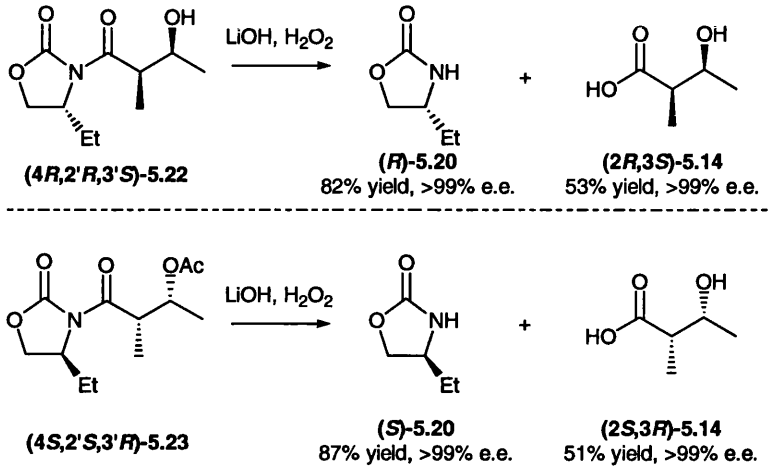
The application of our methodology produced resolved alcohol **(4*R*,2'*R*,3'*S*)-5.22** and acetate **(4*S*,2'*S*,3'*R*)-5.23** (Scheme 5.32).



(+/-)-5.22 **(4*R*,2'*R*,3'*S*)-5.22** >99% e.e. **(4*S*,2'*S*,3'*R*)-5.23** 50% conv, >99% e.e.

Scheme 5.32. Enzymatic resolution of aldols **5.22**

Oxidative cleavage afforded enantiomerically enriched **(2*R*,3*S*)-5.14** and **(2*S*,3*R*)-5.14** and the enantiomerically enriched 4-ethyl-2-oxazolidinones **(*R*)-5.20** and **(*S*)-5.20**[39] (Scheme 5.33).



Scheme 5.33. Preparation of enantiomerically enriched 4-ethyl-2-oxazolidinones and 3-hydroxy-2-methylbutanoic acids

We were very pleased to find out that, even when a less hindered 4-substituted oxazolidinone was used, our approach proved to be successful.

5.6 Conclusions

In summary, after examining a range of experimental conditions and experiencing progressive improvements in our methodology, we were able to achieve the aims of the project.

Starting from racemic Evans' auxiliary, by means of a diastereoselective aldol reaction coupled with a lipase-catalysed resolution, we proved that we could achieve the preparation of enantiomerically enriched β-hydroxy acids and enantiomerically enriched 2-oxazolidinones.[40]

The great achievement of our project was the indirect resolution of racemic Evans' auxiliaries.

The choice of Evans' auxiliary can be limited by the availability of an appropriate enantiomerically pure amino acid or alcohol. Aside from the (*S*)-substituted 2-oxazolidinones readily available from naturally occurring α-amino acids such as L-valine and L-phenylalanine,[41] their configurational antipodes are less accessible.

The sterically constrained, designed chiral 2-oxazolidinones, such as 4,5-disubstituted-2-oxazolidinones, conformationally fixed by bicyclo [2.2.1] and bicyclo [2.2.2] systems,[42] are also less easily available.

However, our methodology allows the use of not only racemic amino acids but also racemic amino alcohols to access the otherwise difficult to prepare antipodes. It also offers, for the first time in chiral auxiliary-based chemistry, the possibility of using designed racemic auxiliaries, instead of the difficult to prepare chiral equivalents.

Not only can resolved aldol adducts be obtained using this methodology, but in addition the procedure acts as in indirect enzyme-catalysed resolution of Evans' auxiliaries.

5.7 References

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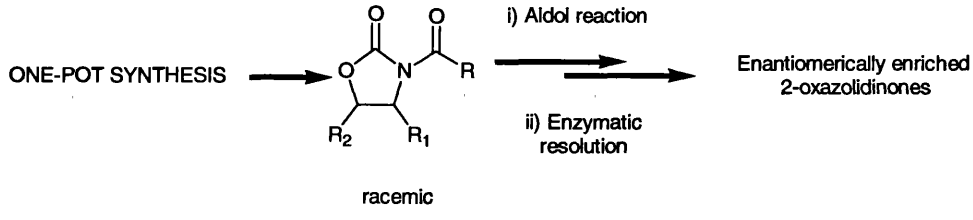
6. Towards the One-Pot Synthesis of *N*-acyl Oxazolidinones

6.1 Introduction

This chapter describes research carried out towards the one-pot synthesis of *N*-acyl oxazolidinones. Despite the high versatility of chiral 2-oxazolidinones through numerous elegant syntheses developed by Evans and others,[1-5] their broader application in asymmetric synthesis is seriously limited by the lack of an easy, safe and low-cost synthesis.

We envisaged that the development of a facile one-pot synthesis of racemic *N*-acyl oxazolidinones could be achieved.

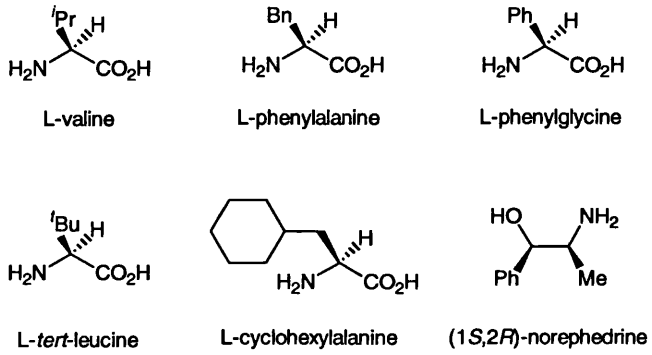
Thereby we would obtain enantiomerically enriched oxazolidinones and be in a position to exploit our methodology of coupling a highly asymmetric aldol reaction to an enzymatic resolution (for a more detailed description, see previous chapter), as illustrated in Scheme 6.1.



Scheme 6.1. General approach to the one-pot synthesis of *N*-acyl oxazolidinones

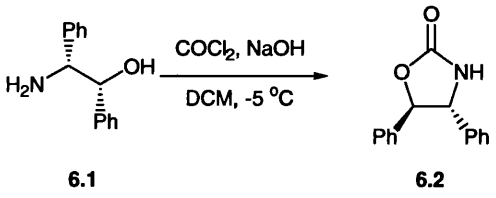
6.2 Background. Synthesis of *N*-acylated Evans auxiliaries

The most common method for the preparation of chiral Evans auxiliaries is the generation of 2-oxazolidinones from β-amino alcohols, in turn usually prepared by reduction of the corresponding amino acids. This procedure provides easy access to enantiopure 2-oxazolidinones from a variety of natural α-amino acids such as L-phenylalanine, L-valine, and L-phenylglycine. In addition, other amino acid-derived starting materials can be employed.[6] Some examples are illustrated in Scheme 6.2.



Scheme 6.2. Oxazolidinone precursor

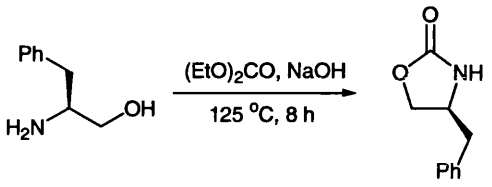
A large number of reagents have been employed in the synthesis of oxazolidinones from amino alcohols. Reactions between amino alcohols and either phosgene or diethyl carbamate or chloroformate represent the most direct route to oxazolidinones. Early methods tended to employ phosgene.[7-10] This method, although a facile one-pot synthesis, is quite hazardous (Scheme 6.3).



Scheme 6.3. Synthesis of 2-oxazolidinones with phosgene

More recently, without the use of phosgene, efficient and high-yielding syntheses of oxazolidinones have been achieved *via* carbamate intermediates.

Using this method, a variety of 4-substituted-2-oxazolidinones such as **6.4** may be synthesised from the corresponding amino alcohol **6.3** with diethyl carbonate under base catalysis (Scheme 6.4)[11,12]

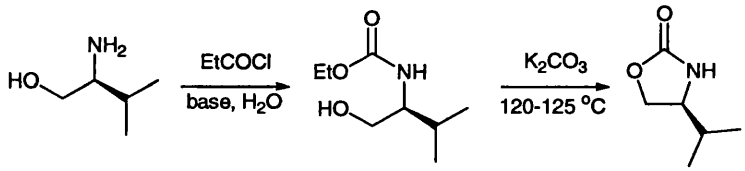


6.3 **6.4**

Scheme 6.4. Synthesis of 2-oxazolidinones with diethyl carbonate

The disadvantages of this method are that the reaction time is around 8 hours and the ethanol by-product must be distilled from the reaction mixture.

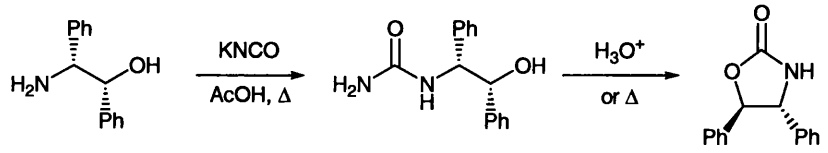
Methyl or ethyl chloroformates can also be used. These first react with the amino group to give the carbamate **6.6**, which is then cyclised in the presence of a base to give the desired oxazolidinone **6.7** (Scheme 5).[5]



6.5 **6.6** **6.7**

Scheme 6.5. Synthesis of 2-oxazolidinones with chloroformate

Other traditional methods include the preparation of enantiomerically pure 2-oxazolidinones **6.2** with isocyanate (Scheme 6.6).[6]

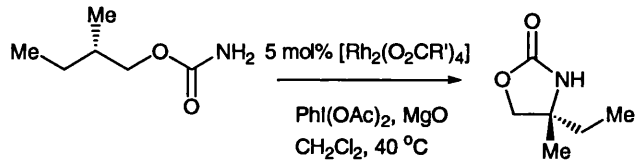


6.1 **6.8** **6.2**

Scheme 6.6. Preparation of 2-oxazolidinones with isocyanate

New approaches to an easy, low cost and high yielding synthesis of enantiomerically enriched 2-oxazolidinones have been explored in the last few years.

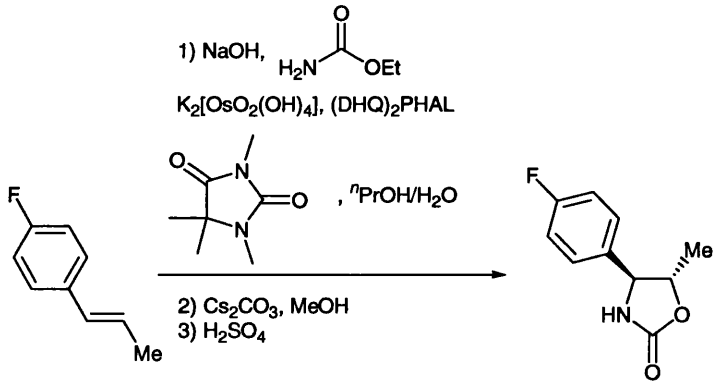
One of these approaches is the oxidative conversion of a carbamate **6.9** to disubstituted 2-oxazolidinone **6.10** by a Rh-catalysed C-H insertion reaction (Scheme 6.7).[13] This methodology utilizes catalytic quantities of a Rh (II) carboxylate complex and an inexpensive commercial oxidant, $PhI(OAc)_2$.



6.9 **6.10**

Scheme 6.7. Synthesis of 2-oxazolidinones by a Rh-catalysed C-H insertion reaction

A one-pot preparation of enantiomerically enriched 4,5-disubstituted-2-oxazolidinones using a modified Sharpless asymmetric dihydroxylation has also been reported (Scheme 6.8).[14] This method utilizes an asymmetric aminohydroxylation of β-substituted styrene derivatives, such as compound **6.11**, followed by base-mediated ring closure.

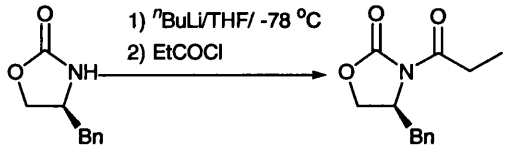


6.11 **6.12**

71% yield, 90-93% e.e.

Scheme 6.8. One-pot preparation of chiral 4,5-disubstituted-2-oxazolidinones *via* modified Sharpless asymmetric aminohydroxylation

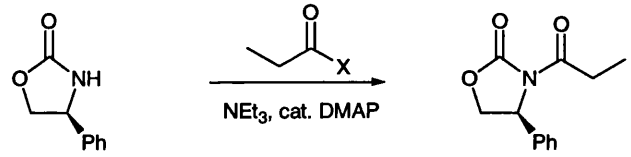
Since the majority of chiral auxiliary-based reactions (alkylations, α-substitution, aldol reactions) are performed on *N*-acyl oxazolidinones, at least another step has to be added to the above-described reactions, for the chiral auxiliary to be synthetically useful. *N*-Acylation of enantiopure oxazolidinones is usually achieved by deprotonation of the auxiliary **6.4** with *n*-butyllithium, followed by the addition of acid chloride (Scheme 6.9).[7,15,16]



6.4 **6.13**

Scheme 6.9. *N*-Acylation with ^{n}BuLi

More recently, a simplified procedure has been developed. The acylation reaction occurs at room temperature in the presence of triethylamine and catalytic quantities of 4-(*N*,*N*-dimethylamino)pyridine, thereby eliminating the requirement for a strong base such as *n*-butyllithium. Acylation occurs with both symmetrical and mixed anhydrides, as well as acid chlorides. An example of application is reported in Scheme 6.10.[17]



6.14 **6.15**

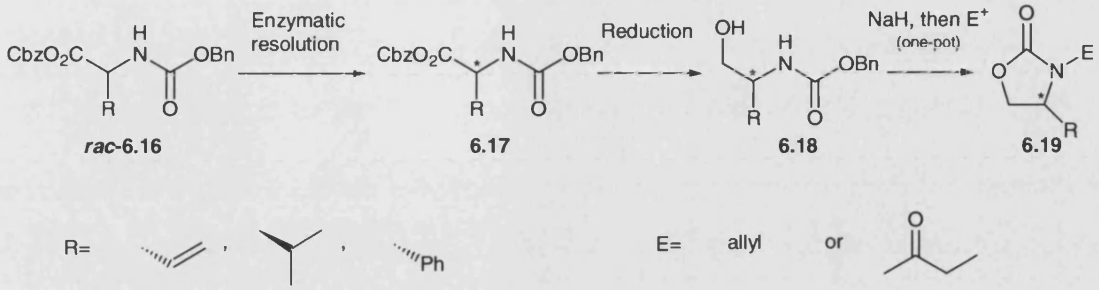
X = OCOEt, OCO^{t}Bu, Cl

Scheme 6.10. *N*-Acylation with NEt_3 and DMAP

In summary, *N*-derivatised-2-oxazolidinones are prepared *via* a process using not less then three steps: amino acid reduction, intermolecular 2-oxazolidinone formation and finally *N*-derivatisation.

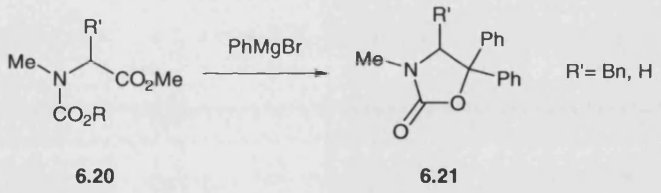
Therefore, considerable research effort has been focused upon the one-pot synthesis of *N*-derivatized-2-oxazolidinones.

In 1994 Huwe and Blechert[18] reported a one-pot synthesis of enantiomerically enriched *N*-acyl and *N*-vinyl oxazolidinones (**6.19**) from amino alcohol carbamates (**6.18**), prepared in their enantiopure forms *via* enzymatic resolution (Scheme 6.11).



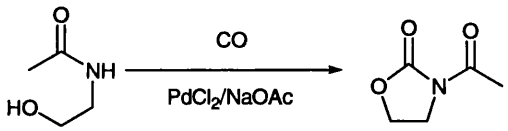
Scheme 6.11. One-pot synthesis of *N*-acyl and *N*-vinyl oxazolidinones from amino alcohol carbamates

N-methyl-oxazolidinone **6.21** has been prepared by the reaction of a Grignard reagent with a *N*-alkoxy-carbonyl-α-amino ester, followed by *in situ* cyclisation (Scheme 6.12).[19] The reaction is however very dependent on the nature of R'. When R' = Ph, no cyclisation occurs; when R' = benzyl or H, the yields are 30 and 60% respectively



Scheme 6.12. One-pot synthesis of *N*-methyl-oxazolidinone **6.21**

N-acetyl-2-oxazolidinone **6.23** has been prepared from the palladium-catalysed carbonylation of *N*-acetyl-β-amino alcohol **6.22** under mild conditions (Scheme 6.13).[20]

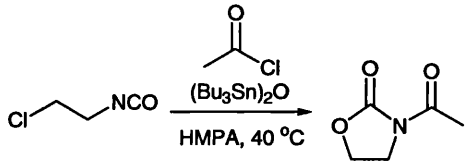


6.22 **6.23**

91% yield

Scheme 6.13. Synthesis of *N*-acetyl-2-oxazolidinone **6.23** by palladium-catalysed carbonylation

The same *N*-acetyl-oxazolidinone was obtained in the reaction between acetyl chloride and an isocyanate (Scheme 6.14).[21]

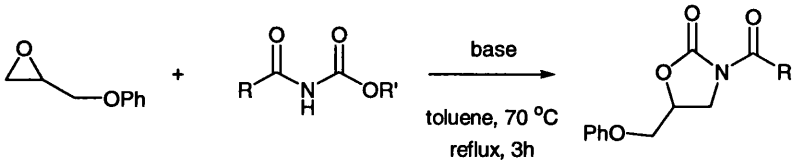


6.24 **6.23**

Scheme 6.14. *N*-acetyl-oxazolidinone **6.23** from an isocyanate

Despite these examples, few of them are of practical use to date.

When we approached this study, our general idea was to find an efficient and easy one-pot synthesis of racemic *N*-acyl oxazolidinones. The application of our methodology described in the previous chapter (the asymmetric aldol reaction coupled to the enzyme-catalysed resolution) would hopefully achieve this goal.
A screening of the literature revealed that in some cases heterocycles, mostly epoxides, had been used to prepare racemic 2-oxazolidinones.[22-28]
We also found an example of one-pot synthesis of 5-substituted *N*-acyl oxazolidinone **6.27** from the reaction of *N*-acylurethane **6.26** with the mono-substituted epoxides **6.25** reported in 1966 (Scheme 6.15).[29]



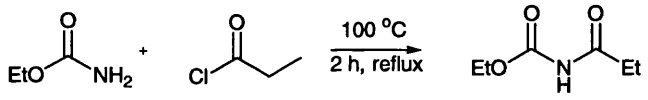
6.25 **6.26** **6.27**

Scheme 6.15. One-pot synthesis of 5-substituted *N*-acyl oxazolidinone **6.27**

We anticipated that this last example would provide a convenient starting point for the development of a one-pot synthesis of 4,5-disubstituted-*N*-acyl oxazolidinones from *N*-acyl urethane **6.26.**

6.3 The one-pot synthesis of *N*-acyl-5-substituted-2-oxazolidinone

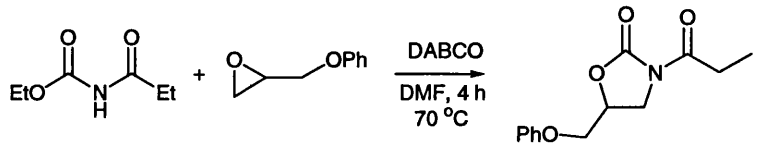
Our study began with the synthesis of a 5-substituted-acyl oxazolidinone previously reported in literature.[29] *N*-acyl urethane **6.28** was prepared from the commercially available and inexpensive acyl chloride **6.29** and ethyl carbamate **6.30.** Heating the two reagents at reflux for 2 hours until HCl evolution ceased gave **6.30** in 58% yield (Scheme 6.16).[30]



6.28 **6.29** **6.30**
58% yield

Scheme 6.16. Synthesis of carbamic acid ester **6.30**

The obtained carbamic ester was then reacted with phenoxymethyl oxirane **6.25,** in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO), to afford *N*-propionyl-5-phenoxymethyl-2-oxazolidinone **6.31** (Scheme 6.17).

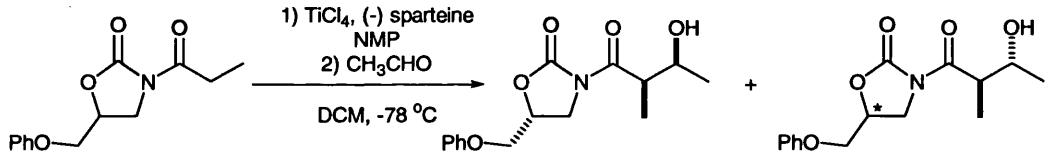


6.30 **6.25** **6.31**
32% yield

Scheme 6.17. One-pot synthesis of *N*-propionyl-5-phenoxymethyl-2-oxazolidinone **6.31**

Disappointingly however, the reaction proceeded in low yield (32% isolated product). The authors noted the same problem. The poor yield was attributed to N→O migration of the acyl group during the course of the reaction.[29]

Despite the disappointing yield, we continued our investigation to examine the subsequent diastereoselective aldol reaction. Reaction with acetaldehyde in the presence of $TiCl_4$, (-)-sparteine and *N*-methyl-2-pyrrolidone[31] provided racemic syn-**6.32** aldol adducts in 31% yield and in low diastereoselectivity (47% d.e.). The formation of other aldol adducts during the reaction was also observed (Scheme 6.18).



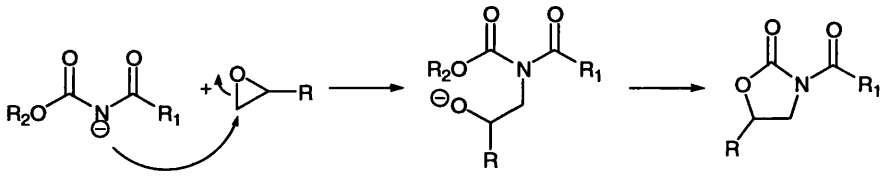
6.31 ***rac-syn*-6.32** **6.32** (other aldol adducts)
31% yield, 47% d.e. 17% yield

Scheme 6.18. Aldol reaction of *N*-propionyl-5-phenoxymethyl-2-oxazolidinone **6.31**

This result was not a surprise. Although high stereoselectivity of the aldol reaction with 4-substituted oxazolidinones is easily achieved, lower diastereoselectivity was expected when a 5-substituted oxazolidinone was used instead. In this case, the controlling stereocentre is too remote from the enolate formed during the reaction to provide high diastereoselectivity.

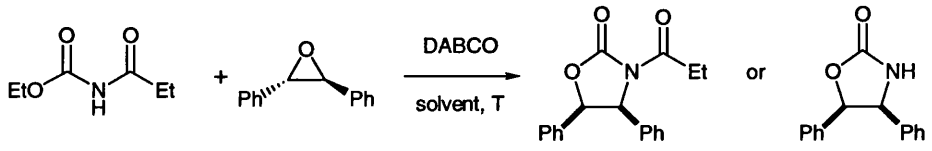
6.4 The attempted one-pot synthesis of *N*-acyl-4,5-disubstituted-2-oxazolidinone

The next step was to attempt the synthesis of an alternative acyl oxazolidinone, which could provide higher diastereoselectivity in the aldol reaction.
The major issue in the reaction between a carbamic ester and a mono-substituted epoxide is that the nucleophile tends to attack the epoxide at less hindered site,[32] giving preferentially 5-substituted-2-oxazolidinones after cyclisation (Scheme 6.19).



Scheme 6.19

With these premises, we couldn't expect to be able to synthesise a 4-substituted oxazolidinone via this methodology. Therefore, we changed approach, examining instead the synthesis of the useful *N*-acylated 4,5-diphenyl-2-oxazolidinone.[33-36]
Trans-stilbene oxide **6.33** was synthesised by epoxidation of *trans*-stilbene.[37] This was then allowed to react with carbamic acid ethyl ester **6.30** in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO) as base. Two products were obtained when this reaction was performed (Scheme 6.20).



6.30 **6.33** ***rac-cis*-6.34** ***rac-cis*-6.35**

Scheme 6.20. One-pot synthesis of *N*-acylated 4,5-diphenyl-2-oxazolidinone **6.34**

The reaction between carbamic acid ester **6.30** and *trans*-stilbene oxide **6.33** was more complicated than had been expected. *Trans*-stilbene oxide turned out to be quite unreactive[38,39] and attempts to increase the temperature and/or increase

base:substrate ratio, led only to deacylated product **6.35** as a consequence of the strong reaction conditions. These results are illustrated in Table 6.1.

Table 6.1. One-pot synthesis of *N*-propionyl-4,5-diphenyl-2 oxazolidinone **6.34**[a]

Entry	Carb. ester 6.30 (eq)	DABCO (eq)	Solvent	T (°C)	Time (h)	Product (% yield)[b]
1	1	0.0004 (0.2%)	DMF	70	7	no reaction
2	1	0.5 (50%)	DMF	150	72	***rac-cis*-6.35** 17%
3	2	0.2 (20%)	PhMe	180	72	***rac-cis*-6.35** 20%
4	2	0.2 (20%)	PhMe	180	3	***rac-cis*-6.34** 22%
5	2	0.2 (20%)	PhMe	150	5	no reaction
6	1	0.2 (20%)	PhMe	180	7	***rac-cis*-6.34** 19% (+ traces of **6.35**)
7	1	0.2 (20%)	DMF	180	7	***rac-cis*-6.35** traces

[a] Reactions were carried out using 1 eq of *trans*-stilbene oxide **6.32** at 3 M concentration.

[b] Yield of the isolated product after flash chromatography.

After several attempts, the best result we could achieve was a 22% yield of racemic ***cis*-6.34** in 3 hours, using 2 equivalents of base in toluene at 180 °C (entry 4). The use of a lower temperature (entries 1 and 5) gave no reaction in 7 h and the starting material was recovered intact. Increase of the reaction time and temperature (entries 2 and 3) resulted only in deacylation of **6.34** to **6.35**, as noted previously.

6.5 Conclusions

Racemic *N*-acylated-5-substituted-2-oxazolidinone was synthesised using a one-pot synthesis from a carbamic acid ester and a monosubstituted epoxide, but the

reaction proceeded in low yield. The subsequent aldol reaction gave racemic syn aldol adducts as major products, but with low diastereoselectivity.

We then attempted a one-pot synthesis of *N*-acyl-4,5-diphenyl-2-oxazolidinone. Unfortunately, the reaction we focused upon gave a lower yield than expected. This may be attributed to the poor nucleophilicity of the carbamic acid ester substrate under the reaction conditions and/or to the poor reactivity of *trans*-stilbene oxide under the same conditions.
Nevertheless, we have demonstrated that there is a scope for successful future attempts with some variations at the one-pot synthesis of *N*-acylated-4,5-disubstituted-2-oxazolidinone.

6.6 References

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7. Experimental

7.1 General experimental

Reactions requiring anhydrous conditions were carried out under an atmosphere of nitrogen. Apparatus, needles and syringes were oven-dried. General solvents used in these reactions were distilled prior to use. Toluene and hexane were distilled from sodium wire. Diethyl ether and THF were distilled from the sodium benzophenone ketyl anion. DCM was distilled from calcium hydride. Cyclohexane, DMF, methanol and ethanol were all purchased in anhydrous form from commercially available suppliers. All solvents used were stored in the presence of activated 3 Å molecular sieves.

Flash chromatography was carried out using Merck Kieselgel 60H and Fisher Matrex Silica 60 silica. TLC was performed using commercially available Merck Kieselgel G/UV_{254} neutral silica coated aluminium and glass plates to monitor all reactions. TLC plates were visualised by 254 nm light or with $KMnO_4$ dip followed by heating.
Organic solvents were dried over anhydrous $MgSO_4$ or Na_2SO_4 and evaporated with a Büchi rotary evaporator. Further drying was carried out on a high-vacuum line when necessary.

Melting points were measured on a Büchi 535 Series instrument and are uncorrected.

Optical rotations were measured with a Optical Activity LTD AA-10 automatic polarimeter.

IR spectra were recorded as thin films, solutions (CH_2Cl_2, $CHCl_3$ or $CDCl_3$) or KBr discs, using a Perkin-Elmer 1600 Series FT-IR spectrophotometer in the range of 4000-600 cm^{-1}, with internal background scan.

Proton ^1H NMR spectra were run in $CDCl_3$, unless otherwise stated, using either a Bruker AM-300 (300 MHz) or a Jeol GX400 (400 MHz) instrument. Chemical shift are reported relative to Me_4Si (δ 0.00 ppm) as internal standard. Coupling constants (J) are given in Hertz and multiplicities denoted as singlet (s), doublet (d), triplet (t), doublet of doublets (dd), doublet of quartets (dq) or multiplet (m). Carbon ^{13}C NMR spectra were run in $CDCl_3$, unless otherwise stated, using either a Bruker AM-300 (75.5 MHz) or a Jeol GX400 (100 MHz) instrument.

Mass spectra, including high-resolution spectra, were recorded on a Fisons NG-Micromass Autospec Spectrometer using electron impact (EI+) ionisation, chemical impact (CI+) ionisation and/or fast atom bombardment (FAB+) ionisation.

Elemental analyses were performed using a Carlo Erba 1106 Elemental Analyser at the University of Bath.

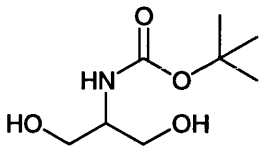
HPLC was performed using a *tsp* liquid chromatograph (Spectra Series P200 Pump, Spectra Series UV100 Lamp, ChromJet SP4400 Integrator). All separations were carried out using Chiralcel OD® and AD® columns obtained from Fisher Scientific Supplies.

Candida antarctica lipase type B (CAL B, Chirazyme® L-2, carrier-fixed C3, lyophilizate) was a gift from Boeringer Mannheim. Porcine pancreas lipase (PPL, EC.3.1.1.3. type II) and *Aspergillus oryzae* protease (type XXII) were from Sigma. *Pseudomonas cepacia* lipase (PCL) and *Pseudomonas fluorescens* lipase (PFL) immobilised in Sol-Gel-AK, *Candida cylindracea* lipase (CCL), *Mucor javanicus* (MJL) lipase and *Aspergillus niger* lipase (ANL) were from Fluka. *Bacillus subtilis* proteinase, *Escherichia coli* Penicillum amidase and hog liver esterase immobilised on Eupergit C were also from Fluka.

Unless preparative details are provided, all chemicals were commercially available and purchased from Acros, Fluka, Lancaster or Sigma-Aldrich.

7.2 Experimental for Chapter 3

Preparation of *N*-(*tert*-butoxycarbonyl)-1,3-propanediol (*N*-Boc-serinol) 3.1[1]



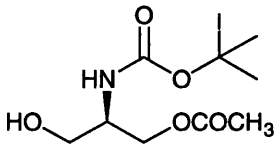
Di-*tert*-butyl dicarbonate (1.31 g, 6.00 mmol) was added slowly to a stirred solution of serinol (2-amino-1,3-propanediol) **3.20** (0.456 g, 5.00 mmol) in EtOH (15 mL). After 1 hour at room temperature, the solvent was removed under reduced pressure. The residue was extracted with EtOAc (2 x 15 mL), and the organic extract was dried (Na_2SO_4) and concentrated *in vacuo*. The obtained white solid was recrystallised from hexane/EtOAc to give **3.1** (0.908 g, 95%) as white needles; mp 84-85 °C (Lit.[1] 87-88 °C); HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 95:5, 1 mLmin^{-1}, λ = 210 nm): t_R = 8.6 min; IR (CH_2Cl_2): ν = 3425, 2965, 2885, 1700, 1500, 1165, 1050 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 5.25 (1H, br d, *J* = 5.4 Hz, N*H*), 3.82-3.63 (5H, m, 2 x C*H*$_2$OH and C*H*N), 2.68 (2H, t, *J* = 5.5 Hz, 2 x CH$_2$O*H*), 1.45 (9H, s, 3 x C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 156.8 (*C*O), 80.3 (*C*(CH$_3$)$_3$), 63.2 (2 x *C*H$_2$OH), 53.5 (*C*HN), 28.7 (3 x *C*H$_3$); Anal. calcd. for $C_8H_{17}NO_4$: C 50.25%, H 8.96%, N 7.32%; found: C 50.2%, H 8.9%, N 7.2%.

General procedure for the enzymatic desymmetrisation of *N*-Boc-serinol 3.1

In a typical experiment a solution of *N*-Boc-serinol **3.1** (0.688 g, 3.59 mmol) and vinyl acetate (2.6 mL, 28.8 mmol), in organic solvent (20 mL) was stirred with the lipase (0.500 g, 140 $mg_{enzyme}/mmol_{sub}$) at different temperatures for 2 hours. Aliquots were taken from the different solutions and analysed by chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 95:5, 1 mLmin^{-1}, λ = 210 nm). Conversion (%), enantiomeric excess (%) and mono/di-acetylated product ratio obtained using different solvents, lipases and temperatures are illustrated in Tables 3.1, 3.2 and 3.3. The reaction performed in vinyl acetate at 30 °C, using PPL as catalyst was followed until completion was reached (2 hours). The reaction was then stopped by filtering the solution. The solution was concentrated *in vacuo* and the residue purified by flash

chromatography (SiO_2, *n*-hexane/EtOAc 50:50) to give **(*R*)-3.2** as a colourless oil (0.580 g, 69% isolated yield) and traces of **3.21** (0.030 g, 3%) as a yellow oil.

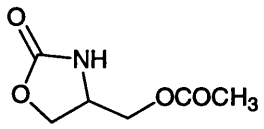
(*R*)-(+)-3-*O*-Acetyl-2-*N*-(*tert*-butoxycarbonyl)serinol (*R*)-3.2



Desymmetrised serinol **(*R*)-3.2** was obtained as a colourless oil (0.580 g, 69% isolated yield); R_f (*n*-hexane/EtOAc 50:50) = 0.29; $[\alpha]_D^{30}$ = +3.5 (*c* 0.56, $CHCl_3$); HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 95:5, 1 mLmin^{-1}, λ = 210 nm): t_R = 10.8 min; IR (film): ν = 3370 (br), 2975, 1710, 1690, 1525, 1370, 1240, 1170, 1040 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 5.10 (1H, br d, *J* = 8.4 Hz, N*H*), 4.19 (2H, d, *J* = 5.7 Hz, CH_2OCO), 3.98-3.88 (1H, m, C*H*N), 3.68 (1H, dd, *J* = 4.4 and 11.4 Hz, CH*H*OH), 3.62 (1H, dd, *J* = 5.0 and 11.4 Hz, C*H*HOH), 3.02 (1H, br s, O*H*), 2.09 (3H, s, COCH_3) 1.45 (9H, s, 3 x CCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 171.8 (O*C*O), 155.2 (N*C*O), 80.3 (*C*$(CH_3)_3$), 63.4 (*C*H_2OCO), 62.2 (*C*H_2OH), 51.4 (*C*HN), 28.7 (3 x C*C*H_3), 21.2 (CO*C*H_3); MS (CI+): *m/z* 234 (MH^+, 45%), 178 (100), 160 (53), 134 (62), 118 (60), 102 (72); Anal. calcd. for $C_{10}H_{19}NO_5$: C 51.49%, H 8.21%, N 6.00%; found: C 51.1%, H 8.2%, N 5.9%.

Traces of diacetylated serinol **3.21** (0.030 g, 3%) were obtained as a yellow oil; R_f (*n*-hexane/EtOAc 50:50) = 0.70; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 95:5, 1 mLmin^{-1}, λ = 210 nm): t_R = 9.7 min; IR (CH_2Cl_2): ν = 3360 (br), 2975, 1745, 1715, 1520, 1370, 1230, 1165, 1045 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 4.90 (1H, br s, N*H*), 4.19-4.08 (5H, m, 2 x CH_2OCO and C*H*N), 2.08 (6H, s, 2 x COCH_3) 1.45 (9H, s, 3 x CCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 168.7 (2 x O*C*OCH_3), 153.3 (N*C*O), 80.0 (*C*$(CH_3)_3$), 61.2 (2 x *C*H_2O), 46.4 (*C*HN), 26.3 (3 x C*C*H_3), 18.8 (CO*C*H_3); MS (CI+): *m/z* 276 (MH^+, 29%), 220 (100), 202 (35), 176 (65), 160 (66), 102 (61).

Preparation of (*R/S*)-(+/-)-4-acetoxymethyl-2-oxazolidinone *rac*-3.3[2]



Powdered K_2CO_3 (0.010 g, 0.07 mmol) was added to desymmetrised serinol **(*R*)-3.2** (0.676 g, 2.89 mmol) and the mixture was heated to 130 °C with magnetic stirring under vacuum, until the gas evolution stopped (approximately 2 hours). After cooling down to room temperature, the mixture was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 10:90), to give ***rac*-3.3** as a colourless oil. Recrystallisation from *n*-hexane/isopropanol afforded ***rac*-3.3** as a white solid (0.263 g, 57%); mp 77-79 °C; R_f (*n*-hexane/EtOAc 10:90) = 0.41; HPLC (Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1 $mLmin^{-1}$, λ = 210 nm): t_R = 16.3 and 18.8 min; IR (film): ν = 3435, 2950, 1720, 1660, 1450, 1370, 995 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 5.71 (1H, br s, N*H*), 4.51 (1H, dd, app. t, *J* = 8.5 Hz, C*H*HO), 4.24-4.01 (4H, m, CH_2OCOCH_3, CH*H*O, C*H*N), 2.11 (3H, s, $COCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 170.8 (O*C*O), 159.8 (N*C*O), 66.9 (CH_2OCOCH_3), 64.9 (CH_2OCON), 51.1 (*C*HN), 20.7 ($COCH_3$); MS (CI+): *m/z* 160 (MH^+, 100%), 118 (23), 99 (20), 86 (30); Anal. calcd. for $C_6H_9NO_4$: C 45.28%, H 5.70%, N 8.80%; found: C 45.3%, H 5.7%, N 8.6%.

General procedure for the enzymatic hydrolysis of (*R/S*)-(+/-)-4-acetoxymethyl-2-oxazolidinone *rac*-3.3

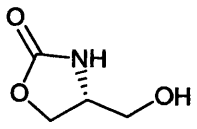
In a typical experiment a solution of ***rac*-3.3** (0.095 g, 0.60 mmol) in aqueous phosphate buffer (1.5 mL, 0.1 M, pH 7) was stirred at 30 °C with the lipase (0.084 g, 140 $mg_{enzyme}/mmol_{substrate}$). After 8 hours, the reaction was stopped by filtering off the enzyme. The aqueous phase was extracted with ethyl acetate (3 x 2 mL) and the combined organic extracts were concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc/MeOH, 90:10) to give **(*R*)-3.22** as a white solid. Yields and specific rotations [Lit.[3] $[\alpha]_D^{30}$ = +32.2 (*c* 1.04, MeOH)] of the products obtained using different lipases are reported in Table 7.1.

Table 7.1

Lipase	mmol	Yield (%)	$[\alpha]_D^{30}$ (*c* 1.0, MeOH)	Optical purity[a] (%)
CAL B	0.20	33	+30.0	93
PCL	0.26	36	+27.0	90
PPL	0.25	42	+29.0	84

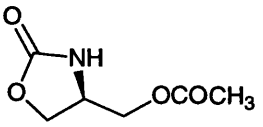
[a] Optical purity= {$[\alpha]_D^{30}$ of obtained (*R*)-4}/{$[\alpha]_D^{30}$ of enantiopure (*R*)-4} x 100

(*R*)-(+)-4-Hydroxymethyl-2-oxazolidinone (*R*)-3.22[3]



Hydroxymethyl oxazolidinone **(*R*)-3.22** was obtained as a white solid; mp 95-97 °C (Lit.[3] 96-99 °C); R_f (EtOAc/MeOH, 90:10) = 0.38; ^{1}H NMR (300 MHz, D_2O): δ_H = 4.50 (1H, dd, app. t, *J* = 9.0, CH*H*OCON), 4.23 (1H, dd, *J* = 9.0, 5.2, C*H*HOCON), 4.04-3.97 (1H, m, C*H*N), 3.61 (1H, dd, *J* = 12.0 and 3.7, CH*H*OH), 3.52 (1H, dd, *J* = 12.0 and 4.1 Hz, C*H*HOH).

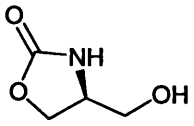
Preparation of (*S*)-(-)-4-acetoxymethyl-2-oxazolidinone (*S*)-3.3[4,5]



To a stirred solution of **(*R*)-3.2** (0.700 g, 3.00 mmol) in THF (90 mL) was added thionyl chloride (1.8 mL, 24.6 mmol) at room temperature under a nitrogen atmosphere. After the solution was heated at reflux for 5 hours, the reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc/hexane 90:10). Recrystallisation from *n*-hexane/isopropanol afforded **(*S*)-3.3** as a white solid (0.343 g, 72%); mp 77-79 °C; R_f (*n*-hexane/EtOAc 10:90) = 0.41; HPLC (Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1 mLmin^{-1}, λ = 210 nm):

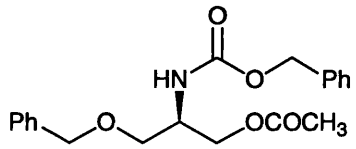
t_R = 18.8 min; $[\alpha]_D^{30}$ = -40.7 (*c* 1.35, $CHCl_3$); IR (CH_2Cl_2): ν = 3435, 2950, 1720, 1660, 1450, 1370, 995 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 5.71 (1H, br s, N*H*), 4.51 (1H, dd, app. t, *J* = 8.5 Hz, C*H*HOCON), 4.24-4.01 (4H, m, CH_2OCOCH_3, CH*H*OCON, C*H*N), 2.11 (3H, s, $COCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 170.8 (O*C*O), 159.8 (N*C*O), 66.9 (CH_2OCOCH_3), 64.9 (CH_2OCON), 51.1 (*C*HN), 20.7 ($COCH_3$); HRMS (FAB+): calcd. for $C_6H_9NO_4$ 159.0532, found 159.0535; Anal. calcd. for $C_6H_9NO_4$: C 45.28%, H 5.70%, N 8.80%; found: C 45.3%, H 5.7%, N 8.6%.

Preparation of (*S*)-(-)-4-hydroxymethyl-2-oxazolidinone (*S*)-3.22



A solution of **(*S*)-3.3** (0.033 g, 0.21 mmol) in aqueous phosphate buffer (0.5 mL, 0.1 M, pH 7) was stirred at 30 °C with CAL B (0.028 g, 140 $mg_{enzyme}/mmol_{sub}$) for 24 hours. The aqueous layer was extracted with EtOAc (3 x 1 mL) and the combined organic extracts were concentrated *in vacuo*. Flash chromatography purification (SiO_2, EtOAc/MeOH, 90:10) afforded **(*S*)-3.22** as a white solid (0.018 g, 77%); $[\alpha]_D^{30}$ = -30.0 (*c* 0.6, MeOH); spectroscopic data were in accordance with those above described for **(*R*)-3.22**.

Preparation of (*R*)-(-)-1-*O*-benzyl-3-*O*-acetyl-2-*N*-(benzyloxycarbonyl)serinol (*R*)-3.23[6-8]

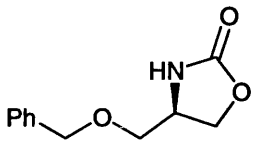


To a stirred solution of **(*R*)-3.2** (1.4 g, 6.00 mmol) in cyclohexane/DCM (60 mL, 50:50) under nitrogen, benzyl-2,2,2,-trichloroacetimidate (3.3 mL, 17.7 mmol) was added. The reaction mixture was stirred with trifluoromethansulfonic acid (54 μL, 0.61 mmol, 10%) at -10 °C for 2 hours, warmed to 0 °C and then left warming to room temperature overnight. The reaction mixture was filtered, quenched with saturated $NaHCO_3$ solution (20 mL), and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried

($MgSO_4$), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc/$CHCl_3$ 70:20:10) to give **(*R*)-3.23** (1.2 g, 55%) as a colourless solid, mp 119-121 °C; R_f (SiO_2, hexane/EtOAc/$CHCl_3$ 70:20:10) = 0.31; HPLC (Chiralcel OD® column, *n*-hexane/isopropanol 90:10, 1 mLmin^{-1}, λ = 254 nm): t_R = 8.7 min; $[\alpha]^{30}$ = -5.2 (*c* 3.25, $CHCl_3$); IR (CH_2Cl_2): ν = 3365 (br), 1711 (br), 1525, 1235 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.36-7.26 (10H, m, 2 × C_6H_5), 5.19 (1H, d, *J* = 8.2 Hz, N*H*), 5.10 (2H, s, CO_2CH_2Ph), 4.52 (1H, d, *J* = 12.2 Hz, CH_2OCHHPh), 4.48 (1H, d, *J* = 12.1 Hz, CH_2OCHHPh), 4.24-4.03 (3H, m, CH_2OCOCH_3, CHN), 3.58 (1H, dd, *J* = 3.3 and 9.3 Hz, CHHOCH$_2$Ph), 3.50 (1H, dd, *J* = 4.7 and 9.3 Hz, CHHOCH$_2$Ph), 1.99 (3H, s, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 170.8 (O*C*O), 155.5 (N*C*O), 137.2, 135.9, 128.2 (2C), 128.1 (2C), 127.9, 127.8, 127.5 (2C), 127.4 (2C) (aromatic C), 73.1 (CH_2OCH_2Ph), 68.4 (CH_2OCH_2Ph), 66.7 (CO_2CH_2Ph), 63.2 (CH_2OCOCH_3), 49.5 (*C*HN), 21.0 ($COCH_3$); MS (CI+): *m/z* 358 (MH^+, 25%), 314 (31), 181 (42), 161 (73), 91 (100).

Compound **3.24** was obtained in traces (<1%) as a yellow oil; R_f (SiO_2, *n*-hexane/EtOAc/$CHCl_3$ 70:20:10) = 0.44; IR (CH_2Cl_2): ν = 3345, 2970, 2930, 1740, 1711, 1510, 1365, 1235, 1165, 1045 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.26 (5H, m, C_6H_5), 4.93 (1H, d, *J* = 7.9 Hz, N*H*), 4.53 (1H, d, *J* = 12.2 Hz, CH_2OCHHPh), 4.49 (1H, d, *J* = 12.1 Hz, CH_2OCHHPh), 4.23-4.03 (3H, m, CH_2OCOCH_3, CHN), 3.56 (1H, dd, *J* = 3.4 and 9.4 Hz, CHHOCH$_2$Ph), 3.48 (1H, dd, *J* = 5.2 and 9.4 Hz, CHHOCH$_2$Ph), 2.01 (3H, s, $COCH_3$), 1.44 (9H, s, 3 × CCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 171.2 (O*C*O), 155.7 (N*C*O), 138.1, 128.9 (2C), 128.5, 128.2 (2C) (aromatic C),80.3 ($C(CH_3)_3$), 73.6 (CH_2OCH_2Ph), 69.1 (CH_2OCH_2Ph), 63.9 (CH_2OCOCH_3), 49.5 (*C*HN), 28.7 ($C(CH_3)_3$), 21.2 ($COCH_3$); MS (CI+): *m/z* 324 (MH^+, 29%), 268 (68), 224 (100), 91 (33).

Preparation of (*R*)-(+)-4-benzyloxymethyl-2-oxazolidinone 3.25



Powdered K_2CO_3 (0.010 g, 0.07 mmol) and water (8 μL, 0.44 mmol, 20%) were added to **3.23** (0.725 g, 2.02 mmol). The mixture was heated to 150 °C with magnetic stirring under vacuum, until the gas evolution stopped. After cooling down to room temperature, the mixture was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane, 65:35), to afford **3.25** (0.264 g, 63%) as a cream solid; mp 49-51 °C; R_f (SiO_2, EtOAc/*n*-hexane 65:35) = 0.34; HPLC (Chiralcel OD® column, *n*-hexane/isopropanol 75:25, 1 mLmin^{-1}, λ = 254 nm): t_R = 22.0 min; $[\alpha]_D^{30}$ = +25.0 (*c* 0.08, $CHCl_3$); IR (CH_2Cl_2): ν = 3450, 2865, 1760, 1400, 1225, 1098 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 5.28 (1H, br s, N*H*), 4.54 (2H, s, CH_2OCH_2Ph) 4.47 (1H, dd, app. t, *J* = 8.4 Hz, C*H*HOCO), 4.13-4.02 (2H, m, CH*H*OCO, C*H*N), 3.50-3.46 (2H, m, CH_2OCH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 160.0 (N*C*O), 137.3, 128.8 (2C), 128.3, 128.0 (2C) (aromatic C), 73.9 (CH_2OCH_2Ph), 72.1 (CH_2OCH_2Ph), 67.3 (CH_2OCO), 52.2 (*C*HN); MS (CI+): *m/z* 208 (MH^+, 100%), 174 (21), 91 (36); HRMS (FAB+): calcd. for $C_{11}H_{13}NO_3$ 207.0895, found 207.0904; Anal. calcd. for $C_{11}H_{13}NO_3$: C 63.76%, H 6.32%, N 6.76%; found: C 64.1%, H 6.3%, N 6.4%.

7.3 Experimental for Chapter 4

General procedure for the lipase-catalysed *N*-acylation of (*R/S*)-(+/-)-4-benzyl-2-oxazolidinone *rac*-4.24

In a typical experiment a solution of ***rac*-4.24** in organic solvent (diisopropyl ether, *n*-hexane, dioxane, toluene, vinyl acetate, vinyl propionate, diisopropyl ether/water, *n*-hexane/isopropanol or *n*-hexane/water) was stirred with the enzyme and the acylating agent (vinyl propionate, vinyl acetate, ethyl propionate, substrate/acyl donor molar ratio 1:1-1:10) at 40 °C.
Reaction details are illustrated in Table 4.1. All reactions were performed in duplicate.
At different times, aliquots of the reaction mixture were taken, worked up by addition of *n*-hexane/isopropanol, filtered and analysed. Conversion and enantiomeric excess values were monitored by chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 75:25, 1mLmin^{-1}, λ = 254 nm and Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1mLmin^{-1}, λ = 254 nm).

Lipase-catalysed *N*-acylation of (*R/S*)-(+/-)-4-isopropyl-2-oxazolidinone *rac*-4.27, (*R/S*)-(+/-)-4-methyl-2-oxazolidinone *rac*-4.28 and 2-oxazolidinone 4.29

***Rac*-4.27**, ***rac*-4.28** and **4.29** (0.30 mmol) were separately reacted with vinyl acetate in a vinyl acetate solution (1 mL, 3 M), in the presence of *Candida antarctica* lipase B as catalyst (33-167 $mg_{enzyme}/mmol_{substrate}$), for 24 hours at 40 °C.
Reaction details are illustrated in Table 4.2. All reactions were performed in duplicate and monitored by TLC.

General procedure for the lipase-catalysed hydrolysis of (*R/S*)-(+/-)-3-propionyl-4-benzyl-2-oxazolidinone *rac*-4.25

In a typical experiment ***rac*-4.25** (0.005 g, 0.02 mmol, 0.01 M) was dissolved in either tris/HCL (2 mL, 5 mM, pH 7.2) or hepes/HCl (2 mL, 10 mM, pH 7.2) aqueous buffer solutions at 40 °C. The reaction was started by the addition of CAL B or CCL (0.030 g, 1.5 $mg_{enzyme}/mmol_{substrate}$). Reaction details are illustrated in Table 4.3.
After 72 hours, samples were extracted with ethyl acetate, dried over $MgSO_4$, concentrated *in vacuo* and analysed by chiral HPLC (Chiralcel® OD column, *n*-

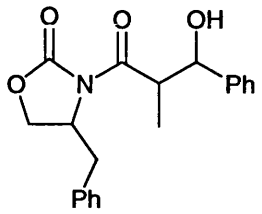
hexane/isopropanol 75:25, 1mLmin^{-1}, λ = 254 nm and Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1mLmin^{-1}, λ = 254 nm).

General procedure for the hydrolysis of (*R/S*)-(+/-)-3-propionyl-4-benzyl-2-oxazolidinone *rac*-4.25 catalysed by other hydrolases

In a typical experiment ***rac*-4.25** (0.070 g, 0.30 mmol) was dissolved in phosphate aqueous buffer (1 mL, 0.01 M, pH 7) at 40 °C. The reaction was started by the addition of the enzyme (protease from *Aspergillus oryzae,* proteinase from *Bacillus subtilis, Penicillum* amidase from *Escherichia coli* or esterase from hog liver immobilised on Eupergit C, 67-133 $mg_{enzyme}/mmol_{substrate}$). Reaction details are illustrated in Table 4.4.
After 48 hours, samples were extracted with ethyl acetate, dried over $MgSO_4$, concentrated *in vacuo* and analysed by chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 75:25, 1mLmin^{-1}, λ = 254 nm and Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1mLmin^{-1}, λ = 254 nm).

7.4 Experimental for Chapter 5

Preparation of *N*-(3-hydroxy-2-methyl-3-phenylpropanoyl)-4-benzyl-2-oxazolidinone 5.7[9]



A solution of lithium diisopropylamide was freshly prepared as follows. To a solution of diisopropylamine (350 μL, 2.50 mmol) in THF (10 mL) at 0 °C under an atmosphere of nitrogen, *n*-BuLi (1.6 M in *n*-hexane, 1.6 mL, 2.50 mmol) was added. After 10 min the solution was cooled to -78 °C and 4-benzyl-3-propionyl-2-oxazolidinone (0.580 g, 2.48 mmol), dissolved in THF (5 mL), was added dropwise over 3 min. After addition was complete, the mixture was stirred at -78 °C for 30 min. To the solution at -78 °C, benzaldehyde (254 μL, 2.50 mmol) was added. After 3-5 s, the reaction mixture was quenched with saturated aqueous NH_4Cl solution (2 mL) and then warmed to room temperature.

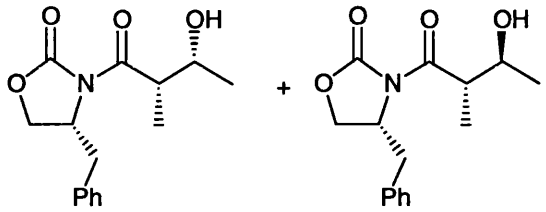
The reaction mixture was diluted with water and extracted with diethyl ether (2 × 10 mL). The organic extracts were washed with 1 M HCl (10 mL), 1 M NaOH (10 mL), and saturated NaCl aqueous solution (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residual mixture of isomers was purified by flash chromatography (*n*-hexane/Et_2O 50:50) to give **5.7** as a yellow oil (0.547 g, 65%); ^1H NMR (400 MHz, $CDCl_3$); δ_H = 7.46-7.25 (10H, m, 2 × C_6H_5), 5.16 (1H, t, J = 3.6 Hz, C*H*OH), 4.70-4.63 (1H, m, C*H*N), 4.19-4.11 (3H, m, CH_2O, COC*H*CH_3), 3.20-3.10 (1H, m, C*H*HPh), 2.87 (1H, d, J = 3.2 Hz, O*H*), 2.65-2.55 (1H, m, CH*H*Ph), 1.17 (3H, d, J = 7 Hz, CH_3). ^1H NMR data were in accordance with those reported in literature.[10]

Enzymatic resolution of 5.7

A mixture of **5.7** (0.050 g, 0.15 mmol) and vinyl acetate (30 μL, 0.32 mmol) in organic solvent (2 mL, *n*-hexane or vinyl acetate) was stirred with *Candida antarctica* lipase type B (0.022 g, 150 $mg_{enzyme}/mmol_{substrate}$) for 72 hours at 40 °C. Periodically aliquots were removed, filtered and diluted in *n*-hexane/isopropanol 80:20, and analysed by

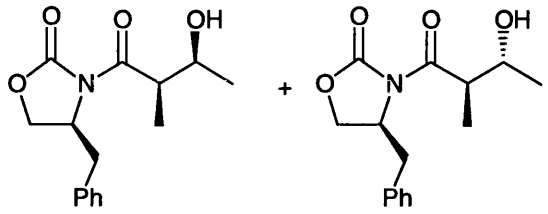
chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 80:20, 1 mLmin^{-1}, λ = 254 nm).

Preparation of (4*R*,2'*S*,3'*R*)- and (4*R*,2'*S*,3'*S*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone 5.8[8]



A solution of $TiCl_4$ in dichloromethane (1 M, 5.4 mL, 5.40 mmol) was added to a stirred solution of 4-benzyl-*N*-propionyl-2-oxazolidinone **(*R*)-5.5** (0.606 g, 2.60 mmol) in dichloromethane (20 mL) at -78 °C under an atmosphere of nitrogen. After 10 minutes, diisopropylethylamine (900 μL, 5.40 mmol) was added, followed 1 hour later by freshly distilled acetaldehyde (300 μL, 5.35 mmol). The reaction mixture was maintained at -78 °C for 5 hours and allowed to warm to room temperature overnight. Saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with DCM (3 x 50 mL). The dichloromethane extracts were washed with water (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, *n*-hexane/EtOAc 50:50) gave a mixture of **(4*R*,2'*S*,3'*R*)-** and **(4*R*,2'*S*,3'*S*)-5.8** as a white solid (0.360 g, 50%, 1:1); mp 89-93 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.63; HPLC (Chiralcel ® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R = 11.0 and 12.2 min; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.36-7.21 (10H, m, 2 x C_6H_5), 4.73-4.65 (2H, m, 2 x NC*H*), 4.24-4.18 (5H, m, 2 x CHCH_2O, C*H*OH), 3.99-3.92 (1H, m, C*H*OH), 3.87-3.77 (2H, m, 2 x COC*H*CH_3), 3.34-3.28 (2H, m, 2 x CH*H*Ph), 2.87 (1H, d, *J* = 2.8 Hz, O*H*), 2.82-2.73 (2H, m, 2 x C*H*HPh), 2.61 (1H, d, *J* = 7.7 Hz, O*H*), 1.30 (3H, d, *J* = 6.3 Hz,COCHCH_3), 1.24 (3H, d, *J* = 6.4 Hz, COCHCH_3), 1.21 (3H, d, *J* = 2.5 Hz, CHOHCH_3), 1.19 (3H, d, *J* = 2.4 Hz, CHOHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 176.6 and 176.7 (2 x C*C*O), 153.4 and 153.5 (2 x O*C*ON), 135.1, 135.2, 129.5 (2C), 129.4 (2C), 129.0 (2C), 128.9 (2C), 127.4, 127.3 (2 x aromatic *C*), 68.1 and 70.6 (2 x *C*HOH), 66.1 and 66.2 (2 x *C*H_2O), 55.4 and 55.6 (2 x *C*HN), 43.0 and 45.0 (2 x CO*C*HCH_3), 37.8 and 38.0 (2 x *C*H_2Ph), 19.5 and 21.2 (2 x CHOH*C*H_3), 10.6 (2 x COCH*C*H_3).

Preparation of (4*S*,2'*R*,3'*S*)- and (4*S*,2'*R*,3'*R*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone 5.8[11]



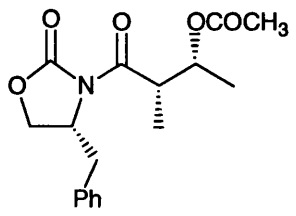
Hydroxy oxazolidinones **(4*S*,2'*R*,3'*S*)-** and **(4*S*,2'*R*,3'*R*)-5.8** were synthesised using the same quantities and following the procedure above described for the synthesis of **(4*R*,2'*S*,3'*R*)-** and **(4*R*,2'*S*,3'*S*)-5.8**. In this case **(*S*)-5.5** (0.606 g, 2.60 mmol) was reacted with acetaldehyde (300 μL, 5.35 mmol). Purification by flash chromatography (SiO_2, *n*-hexane/EtOAc 50:50) afforded a mixture of **(4*S*,2'*R*,3'*S*)-** and **(4*S*,2'*R*,3'*R*)-5.8** as a white solid (0.389 g, 54%, 1:1); mp 90-95 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.59; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 $mLmin^{-1}$, λ = 254 nm): t_R = 11.6 and 12.8 min; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.36-7.22 (10H, m, 2 × C_6H_5), 4.72-4.66 (2H, m, 2 × NC*H*), 4.24-4.16 (5H, m, 2 × CHCH_2O, C*H*OH), 4.09-3.99 (1H, m, C*H*OH), 3.97-3.78 (2H, m, 2 × COC*H*CH_3), 3.34-3.28 (2H, m, 2 × CH*H*Ph), 2.86 (1H, d, app. s, O*H*), 2.82-2.75 (2H, m, 2 × C*H*HPh), 2.57 (1H, d, *J* = 7.4 Hz, O*H*), 1.31 (3H, d, *J* = 6.6 Hz, COCHCH_3), 1.24 (3H, d, *J* = 6.6 Hz, COCHCH_3), 1.21 (3H, d, *J* = 2.3 Hz, CHOHCH_3), 1.20 (3H, d, *J* = 2.4 Hz, CHOHCH_3);^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 177.1 (2 × C*C*O), 152.9 (2 × O*C*ON), 134.8 (2C), 129.2 (4C), 128.8 (4C), 127.3 (2C) (aromatic *C*), 67.6 (2 × *C*HOH), 66.1 (2 × *C*H_2O), 55.1 (2 × *C*HN), 43.1 (2 × CO*C*HCH_3), 37.8 (2 × *C*H_2Ph), 19.6 (2 × CHOH*C*H_3), 10.6 (2 × COCH*C*H_3).

General procedure for the enzymatic resolution of (4*R*,2'*S*,3'*R*)- and (4*R*,2'*S*,3'*S*)-5.8

In a typical experiment alcohols **(4*R*,2'*S*,3'*R*)-** and **(4*R*,2'*S*,3'*S*)-5.8** (0.055 g, 0.20 mmol) and *Candida antarctica* lipase type B (0.010 g, 50 $mg_{enzyme}/mmol_{substrate}$) in organic solvent (vinyl acetate, *n*-hexane, toluene, dichloromethane or diisopropyl ether, 1 ml) were stirred with vinyl acetate (40 μL, 0.40 mmol) at 40 °C. Periodically aliquots were removed, filtered, diluted in a *n*-hexane/isopropanol 80:20 solution, and analysed by chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1

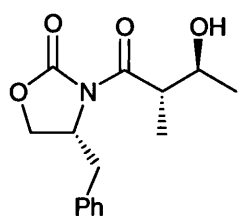
$mLmin^{-1}$, λ = 254 nm). Conversions and enantiomeric excesses obtained in the different solvents at different reaction times are reported in Tables 5.1 and 5.2.
After 24-48 hours, the reaction was stopped by filtering off the enzyme and ester **(4*R*,2'*S*,3'*R*)-5.9** was separated from unreacted alcohol **(4*R*,2'*S*,3'*S*)-5.8** by flash chromatography (SiO_2, *n*-hexane/EtOAc 60:40).

(4*R*,2'*S*,3'*R*)-*N*-(3-acetoxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone
(4*R*,2'*S*,3'*R*)-5.9



Ester **(4*R*,2'*S*,3'*R*)-5.9** was obtained as a yellow oil (0.025 g, 40% yield for the reaction was performed in *n*-hexane); R_f (*n*-hexane/EtOAc, 60:40) = 0.64; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 $mLmin^{-1}$, λ = 254 nm): t_R 8.7 min; $[\alpha]_D^{30}$ = -9.4 (*c* 1.0, $CHCl_3$); IR (film): ν = 1780, 1735, 1695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.36-7.22 (5H, m, C_6H_5), 5.40 (1H, dq, *J* = 4.2 and 6.4 Hz, C*H*OCOCH$_3$), 4.71-4.63 (1H, m, NC*H*), 4.20-4.08 (2H, m, CHCH_2O), 3.91 (1H, dq, *J* = 4.2 and 6.9 Hz, COC*H*CH$_3$), 3.34 (1H, dd, *J* = 3.3 and 13.4 Hz, CH*H*Ph), 2.67 (1H, dd, *J* = 10.3 and 13.4 Hz, C*H*HPh), 2.04 (3H, s, OCOCH_3), 1.33 (3H, d, *J* = 6.4 Hz, COCHCH_3), 1.22 (3H, d, *J* = 6.9 Hz, AcOCHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 174.3 (C*C*O), 170.7 (O*C*OCH$_3$), 153.7 (O*C*ON), 135.9, 129.8 (2C), 129.3 (2C), 127.6 (aromatic *C*), 70.7 (*C*HOCOCH$_3$), 66.6 (*C*H$_2$O), 55.8 (*C*HN), 42.7 (CO*C*HCH$_3$), 38.1 (*C*H$_2$Ph), 21.5 (OCO*C*H$_3$), 18.4 (AcOCH*C*H$_3$), 11.2 (COCH*C*H$_3$); MS (EI+, 70eV): *m/z* 320 (M^{+}+1, 77%), 260 (100), 178 (15), 143 (14), 117.1 (10), 83 (69); Anal. calcd. for $C_{17}H_{21}NO_5$: C 63.93%, H 6.62%, N 4.38%; found: C 63.8%, H 6.6%, N 4.3%.

(4*R*,2'*S*,3'*S*)-*N*-(3-Hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone
(4*R*,2'*S*,3'*S*)-5.8



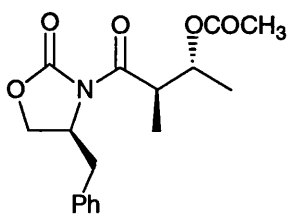
Unreacted alcohol **(4*R*,2'*S*,3'*S*)-5.8** was obtained as a white solid (0.019 g, 35% yield for the reaction was performed in *n*-hexane); mp 74-76 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.63; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R 12.2 min; $[\alpha]_D^{30}$ = -19.8 (*c* 0.5, $CHCl_3$); IR (CH_2Cl_2): ν = 3570 (br), 1780, 1690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.22 (5H, m, C_6H_5), 4.73-4.65 (1H, m, NC*H*), 4.24-4.16 (2H, m, CHCH_2O), 3.96 (1H, dq, app. t, *J* = 6.4 Hz, C*H*OH), 3.83 (1H, dq, *J* = 7.0 and 7.0 Hz, COC*H*CH_3), 3.33 (1H, dd, *J* = 3.3 and 13.4 Hz, CH*H*Ph), 2.79 (1H, dd, *J* = 9.6 and 13.4 Hz, C*H*HPh), 2.55 (1H, br s, O*H*), 1.31 (3H, d, *J* = 6.3 Hz, COCHCH_3),1.20 (3H, d, *J* = 6.9 Hz, CHOHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 176.6 (C*C*O), 153.2 (O*C*ON), 135.2, 129.4 (2C), 128.9 (2C), 127.3 (aromatic *C*), 70.7 (*C*HOH), 66.1 (*C*H_2O), 55.6 (*C*HN), 45.0 (CO*C*HCH_3), 37.8 (*C*H_2Ph), 21.2 (CHOH*C*H_3),14.6 (COCH*C*H_3).

General procedure for the enzymatic resolution of (4*S*,2'*R*,3'*S*)- and (4*S*,2'*R*,3'*R*)-5.8

Enzymatic resolution of **(4*S*,2'*R*,3'*S*)- and (4*S*,2'*R*,3'*R*)-5.8** was carried out as above described for the enzymatic resolution of **(4*R*,2'*S*,3'*R*)- and (4*R*,2'*S*,3'*S*)-5.8**. The reactions in different solvents were followed by chiral HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm). Conversions and enantiomeric excesses obtained in the different solvents at different reaction times are reported in Tables 5.3 and 5.4.

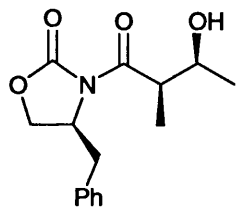
After 24-48 hours, the reaction was stopped by filtering off the enzyme and the acylated oxazolidinone **(4*S*,2'*R*,3'*R*)-5.9** was separated from unreacted alcohol **(4*S*,2'*R*,3'*S*)-5.8** by flash chromatography (SiO_2, *n*-hexane/EtOAc 60:40).

(4*S*,2'*R*,3'*R*)-*N*-(3-Acetoxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*S*,2'*R*,3'*R*)-5.9



Acylated oxazolidinone **(4*S*,2'*R*,3'*R*)-5.9** was obtained as a white solid (0.024 g, 38% yield for the reaction performed in vinyl acetate); mp 89-91 °C; R_f (*n*-hexane/EtOAc, 60:40) = 0.58; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R 14.1 min; $[\alpha]_D^{30}$ = +40.0 (*c* 1.0, $CHCl_3$); IR (CH_2Cl_2) ν = 1790, 1740, 1690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.37-7.21 (5H, m, C_6H_5), 5.22 (1H, dq, *J* = 6.3 and 8.8 Hz, C*H*OCOCH$_3$), 4.74-4.66 (1H, m, NC*H*), 4.23-4.13 (2H, m, CH_2O), 4.07 (1H, dq, *J* = 7.0 and 8.8 Hz, COC*H*CH$_3$), 3.24 (1H, dd, *J* = 3.2 and 13.2 Hz, CH*H*Ph), 2.74 (1H, dd, *J* = 9.5 and 13.2 Hz, C*H*HPh), 2.00 (3H, s, OCOCH_3), 1.31 (3H, d, *J* = 6.3 Hz, COCHCH_3), 1.20 (3H, d, *J* = 7.0 Hz, AcOCHCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 174.5 (C*C*O), 170.0 (O*C*OCH$_3$), 153.0 (O*C*ON),135.0, 129.4 (2C), 129.0 (2C), 127.4 (aromatic *C*), 72.1 (*C*HOCOCH$_3$), 65.8 (*C*H$_2$O), 55.2 (*C*HN), 42.7 (CO*C*HCH$_3$), 37.7 (*C*H$_2$Ph), 21.1 (OCO*C*H$_3$), 17.2 (AcOCH*C*H$_3$), 13.7 (COCH*C*H$_3$); MS (EI+, 70 eV): *m/z* 319 (M^{+}, 70%), 303 (40), 286 (26); 259 (34), 143 (32), 117 (25), 83 (100), 55 (30), 43 (60); Anal.calc. for $C_{17}H_{21}NO_5$: C 63.93%, H 6.62%, N 4.38%; found: C 63.8%, H 6.6%, N 4.4%.

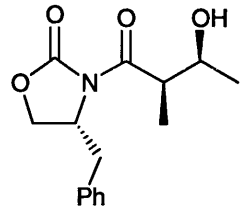
(4*S*,2'*R*,3'*S*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*S*,2'*R*,3'*S*)-5.8



Unreacted alcohol **(4*S*,2'*R*,3'*S*)-5.8** was obtained as a white solid (0.017 g, 30% yield for the reaction performed in vinyl acetate); mp 112-114 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.59; HPLC (Chiralcel ® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm) t_R = 12.8 min; $[\alpha]_D^{30}$ = +42.0 (*c* 0.50, $CHCl_3$); IR (CH_2Cl_2): ν = 3575 (br), 1775, 1690 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.21 (5H, m, C_6H_5), 4.73-4.65 (1H, m, NC*H*), 4.24-4.15 (3H, m, CHCH_2O, C*H*OH), 3.84 (1H, dq, *J* = 3.0 and 7.0 Hz, COC*H*CH$_3$), 3.31 (1H, dd, *J* = 3.4 and 13.4 Hz, CH*H*Ph), 2.86 (1H, d, *J* = 2.5 Hz, O*H*), 2.77 (1H, dd, *J* = 9.6 and 13.4 Hz, C*H*HPh), 1.23 (3H, d, *J* = 6.4 Hz, COCHCH_3), 1.21 (3H, d, *J* = 7.0 Hz, CHOHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 176.7 (C*C*O), 153.4 (O*C*ON), 135.1, 129.4 (2C), 128.9 (2C), 127.4 (aromatic *C*), 68.1

(*C*HOH), 66.1 (*C*H_2O), 55.4 (*C*HN), 43.0 (CO*C*HCH$_3$), 38.0 (*C*H_2Ph), 19.5 (CHOH*C*H$_3$), 10.6 (COCH*C*H3).

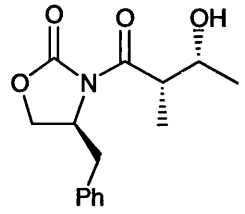
Preparation of (4*R*,2'*R*,3'*S*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.8[12]



$TiCl_4$ (1M solution in DCM, 2.6 mL, 2.58 mmol) was added to a solution of *N*-propionyl-2-oxazolidinone **(*R*)-5.5** (0.576 g, 2.46 mmol) in dichloromethane (20 mL) at 0 °C under an atmosphere of nitrogen. The mixture was stirred for 5 min and (-)-sparteine (560 μL, 2.46 mmol) was added dropwise slowly. After complete addition and 20 min at 0 °C, the mixture was cooled to -78 °C and *N*-methyl-2-pyrrolidinone (240 μL, 2.46 mmol) was added. The reaction mixture was stirred for 10 min, followed by addition of freshly distilled acetaldehyde (240 μL, 4.28 mmol) dropwise. The mixture was allowed to react for 1 h at -78 °C, gradually warmed to 0 °C, and stirred for 1 h. The reaction was quenched with half-saturated NH_4Cl (10 mL) and warmed to room temperature. The layers were separated and the aqueous layer was extracted twice with DCM (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification *via* flash chromatography (SiO_2, *n*-hexane/EtOAc, 50:50) afforded **(4*R*,2'*R*,3'*S*)-5.8** (0.348 g, 51%) as colourless crystals; mp 114-115 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.37; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R = 10.0 min; $[\alpha]_D^{30}$ = -54.5 (*c* 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.36-7.20 (5H, m, C_6H_5), 4.74-4.67 (1H, m, NC*H*), 4.26-4.10 (3H, m, CHCH_2O, C*H*OH), 3.74 (1H, dq, J = 2.7 and 7.0 Hz, COC*H*CH$_3$), 3.25 (1H, dd, *J* = 3.5 and 13.2 Hz, CH*H*Ph), 2.91 (1H, d, *J* = 2.7 Hz, O*H*), 2.79 (1H, dd, *J* = 9.7 and 13.4 Hz, C*H*HPh), 1.27 (3H, d, *J* = 7.0 Hz, COCHCH_3), 1.21 (3H, d, *J* = 6.2 Hz, CHOHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 178.0 (C*C*O), 153.7 (O*C*ON), 135.6, 130.0 (2C), 129.6 (2C), 128.0 (aromatic *C*), 68.2 (*C*HOH), 66.8 (*C*H_2O), 55.7 (*C*HN), 43.7 (CO*C*HCH$_3$), 38.4 (*C*H_2Ph), 20.1 (CHOH*C*H$_3$), 11.1 (COCH*C*H$_3$); MS (FAB+): *m/z* 278 (MH$^+$, 100%); HRMS (FAB+) calcd. for $C_{15}H_{19}NO_4$: 277.1314, found 277.1314; Anal. calcd. for $C_{15}H_{19}NO_4$: C

64.97%, H 6.91%, N 5.05%; found: C 64.3%, H 6.9%, N 5.1%. See Appendices for crystal structure.

Preparation of (4*S*,2'*S*,3'*R*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*S*,2'*S*,3'*R*)-5.8[12]



Hydroxy oxazolidinone **(4*S*,2'*S*,3'*R*)-5.8** was synthesized following the procedure above described for the synthesis of **(4*R*,2'*R*,3'*S*)-5.8**. *N*-Propionyl-2-oxazolidinone **(*S*)-5.5** (0.576 g, 2.46 mmol) was reacted with acetaldehyde (240 μL, 4.28 mmol) in the presence of $TiCl_4$ (1M solution in DCM, 2.6 mL, 2.58 mmol), (-)-sparteine (560 μL, 2.46 mmol) and *N*-methyl-2-pyrrolidinone (240 μL, 2.46 mmol). Purification *via* flash chromatography (SiO_2, hexane/EtOAc, 50:50) afforded **(4*S*,2'*S*,3'*R*)-5.8** as colourless crystals (0.355 g, 52%); mp 113-114 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.37; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 $mLmin^{-1}$, λ = 254 nm) t_R = 16.7 min; $[\alpha]_D^{30}$ = +56.5 (*c* 0.99, $CHCl_3$) [Lit.[10] +60.9 (*c* 0.99, $CHCl_3$)]; ^1H NMR (400 MHz, $CDCl_3$): δ_H = 7.36-7.20 (5H, m, C_6H_5), 4.74-4.68 (1H, m, NC*H*), 4.26-4.16 (3H, m, CHCH_2O, C*H*OH), 3.74 (1H, dq, J = 2.7 and 7.0 Hz, COC*H*CH_3), 3.25 (1H, dd, *J* = 3.1 and 13.2 Hz, CH*H*Ph), 2.93 (1H, d, *J* = 2.3 Hz, O*H*), 2.78 (1H, dd, *J* = 9.3 and 13.2 Hz, C*H*HPh), 1.28 (3H, d, *J* = 7.0 Hz, COCHCH_3), 1.22 (3H, d, *J* = 6.6 Hz, CHOHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C 174.4 (*C*O), 153.1 (O*C*ON), 135.0, 129.4 (2C), 129.0 (2C), 127.5 (aromatic *C*), 67.6 (*C*OH), 66.2 (CH_2O), 55.1 (*C*HN), 43.1 (CO*C*HCH$_3$), 37.8 (CH_2Ph), 19.6 (CHOH*C*H$_3$),10.1 (COCH*C*H$_3$); MS (FAB+): *m/z* 278 (MH^+, 100%); Anal. calcd. for $C_{15}H_{19}NO_4$: C 64.97%, H 6.91%, N 5.05%; found: C 65.0%, H 6.9%, N 5.1%.

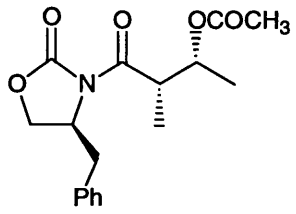
Lipase-catalysed acetylation of alcohol (4*S*,2'*S*,3'*R*)-5.8

Alcohol **(4*S*,2'*S*,3'*R*)-5.8** (0.100 g, 0.36 mmol) and *Candida antarctica* lipase type B (0.010 g, 30 $mg_{enzyme}/mmol_{substrate}$) were stirred with vinyl acetate (1.7 mL) at 40 °C. Periodically aliquots were removed, filtered, diluted in a *n*-hexane/isopropanol 80:20,

and analysed by chiral HPLC. Time course of the enzymatic reaction are reported in Table 5.6.

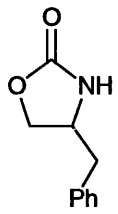
After 72 hours, the reaction was stopped by filtering off the enzyme and acylated oxazolidinone **(4*S*,2'*S*,3'*R*)-5.9** was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc 60:40).

(4*S*,2'*S*,3'*R*)-*N*-(3-Acetoxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*S*,2'*S*,3'*R*)-5.9



Acylated oxazolidinone **(4*S*,2'*S*,3'*R*)-5.9** was obtained as yellow needles (0.086 g, 75%); mp 79-80 °C; R_f (*n*-hexane/EtOAc, 60:40) = 0.66; HPLC (Chiralcel® OD column, n-hexane/isopropanol 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R = 8.2 min; $[\alpha]_D^{30}$ = +77.7 (*c* 0.99, $CHCl_3$); IR (CH_2Cl_2): ν = 2975, 1780, 1735, 1695, 1450, 1370, 1225, 1110 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.36-7.19 (5H, m, C_6H_5), 5.30 (1H, dq, *J* = 4.3 and 6.4 Hz, C*H*OCOCH$_3$), 4.64-4.56 (1H, m, NC*H*), 4.28-4.08 (2H, m, CH_2O), 3.95 (1H, dq, *J* = 4.3 and 6.9 Hz, COC*H*CH$_3$), 3.27 (1H, dd, *J* =3.1 and 13.2 Hz, CH*H*Ph), 2.76 (1H, dd, *J* = 9.7 and 13.3 Hz, C*H*HPh), 2.05 (3H, s, OCOCH_3), 1.28 (3H, d, *J* = 6.5 Hz, COCHCH_3),1.22 (3H, d, *J* = 6.9 Hz, AcOCHCH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 172.2 (C*C*O), 151.7 (O*C*ON), 168.8 (O*C*OCH$_3$), 133.5, 127.6 (2C), 127.1 (2C), 125.5 (aromatic C), 68.4 (*C*HOCOCH$_3$), 64.5 (*C*H$_2$O), 54.0 (N*C*H), 40.5 (CO*C*HCH$_3$), 36.1 (*C*H$_2$Ph), 19.3 (OCO*C*H$_3$), 16.2 (AcOCH*C*H$_3$), 8.9 (COCH*C*H$_3$); MS (EI+, 70eV): *m/z* 319 (M^{+}, 10%), 259 (57), 244 (46), 178 (30), 83 (95), 43 (100); HRMS (EI+) calcd. for $C_{17}H_{21}NO_5$: 319.1420, found 319.1417; Anal. calcd. for $C_{17}H_{21}NO_5$: C 63.93%, H 6.62%, N 4.38%; found: C 63.5%, H 6.6%, N 4.2%.

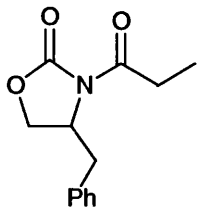
Preparation of (*R/S*)-(+/-)-4-benzyl-2-oxazolidinone *rac*-5.10[2,13]



To a stirred suspension of $NaBH_4$ (5.0 g, 125 mmol) in tetrahydrofuran (50 mL) racemic phenylalanine **5.11** (8.2 g, 49.6 mmol) was added. The flask was immersed in an ice-water bath and a solution of conc. H_2SO_4 (3.3 mL, 61.9 mmol) in diethyl ether (total volume of 10 mL) was added dropwise at such a rate to maintain the reaction temperature below 20 °C. Stirring of the reaction mixture was continued at room temperature overnight. 5 N NaOH (50 mL) was then added and, after removing the solvent that distilled below 100 °C, the mixture was heated at reflux for 3 hours. The turbid aqueous mixture was cooled to room temperature with stirring before introducing $NaHCO_3$ (21.0 g, 250 mmol) in H_2O (40 mL), followed by MeO_2CCl (4.7 mL, 52.5 mmol, cooling with 5 °C bath).

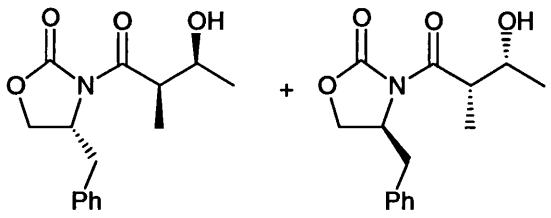
After stirring at room temperature for another 1.5 hours, the mixture was filtered and extracted with EtOAc (4 x 30mL). The combined organic extracts were washed with H_2O (15 mL) and brine (15 mL) and dried over $MgSO_4$. Removal of the solvent gave **5.13** as a white solid. Powdered K_2CO_3 (0.035 g, 0.25 mmol) was added and the mixture was heated to 125 °C with magnetic stirring under vacuum until the gas evolution stopped (approximately 2 hours). The product was dissolved in EtOAc (75 mL) and the organic layer was washed with H_2O (25 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Recrystallysation from *n*-hexane/EtOAc afforded ***rac*-5.10** as a white solid (6.4 g, 72% overall yield); mp 88-90 °C (Lit.[2] 90-91 °C); $[\alpha]_D^{30}$ = 0.0 (*c* 1.0, $CHCl_3$); IR (CH_2Cl_2): ν = 3450, 2920, 1760, 1400, 1250, 1025 cm^{-1}; ^1H NMR (300 MHz, CDCl3): δ_H = 7.37-7.16 (5H, m, C_6H_5), 5.18 (1H, s, N*H*), 4.48 (1H, app. t, *J* = 8.2 Hz, OCH*H*), 4.15 (1H, dd, *J* = 5.6 and 8.4 Hz, OC*H*H), 4.11-4.04 (1H, m, C*H*N), 2.93-2.81 (2H, m, CH_2Ph).

Preparation of (*R/S*)-(+/-)-3-propionyl-4-benzyl-2-oxazolidinone *rac*-5.5[14]



A solution of *n*-BuLi in *n*-hexane (1.6 M, 5.0 mL, 7.96 mmol) was added dropwise, over a period of 10 min, to a stirred solution of ***rac*-5.10** (1.4 g, 7.96 mmol) in anhydrous THF (30 mL) at -78 °C under an inert atmosphere of nitrogen. The mixture was stirred at this temperature for 15 min and EtCOCl (0.7 mL, 7.96 mmol) was then added dropwise over 5 min. After 30 min the reaction quenched with sat. NH_4Cl solution (20 mL) and allowed to warm to room temperature over a 2 hour period. The mixture was extracted with EtOAc (3 × 20 mL) and the organic extracts were combined and washed with brine (20 mL). The dried solution ($MgSO_4$) was concentrated *in vacuo* and the residue was purified by recrystallisation (*n*-hexane/EtOAc) to give ***rac*-5.5** as a colourless crystalline solid (1.7 g, 91%); mp 43-45°C (Lit.[11] 44-46°C); IR (CH_2Cl_2): ν = 2930, 1780, 1700, 1450, 1380, 1215, 1070 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.20 (5H, m, C_6H_5), 4.71-4.63 (1H, m, C*H*N), 4.23-4.14 (2H, m, CH_2O), 3.31 (1H, dd, *J* = 3.3 and 13.3 Hz, CH*H*Ph), 3.04-2.88 (2H, dq app m, CH_2CH_3), 2.76 (1H, dd, *J* = 9.7 and 13.3 Hz, C*H*HPh), 1.20 (3H, t, *J* = 7.3 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C= 175.2 (*C*O), 154.6 (O*C*ON), 136.4, 130.5 (2C), 130.0 (2C), 128.4 (aromatic C), 67.3 (CH_2O), 56.3 (*C*HN), 39.0 (CH_2Ph), 30.3 (CH_2CH_3), 9.4 (CH_3). ^1H and ^{13}C NMR data were in accordance with those reported in literature.[15]

Preparation of (4*R*,2'*R*,3'*S*)- and (4*S*,2'*S*,3'*R*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone *rac-syn*-5.8[12]



Synthesis of ***rac-syn*-5.8** was carried out following the procedure previously described for the synthesis of **(4*R*,2'*R*,3'*S*)-5.8**. *N*-Propionyl-2-oxazolidinone ***rac*-5.5** (0.576 g, 2.46 mmol) was reacted with acetaldehyde (240 μL, 4.28 mmol) in the presence of $TiCl_4$ (1M solution in DCM, 2.6 mL, 2.58 mmol), (-)-sparteine (560 μL, 2.46 mmol) and *N*-methyl-2-pyrrolidinone (240 μL, 2.46 mmol). Purification *via* flash chromatography (SiO_2, hexane/EtOAc, 50:50) afforded ***rac-syn*-5.8** as a white crystalline solid (0.368 g, 54%, >99% d.e.); mp 113-114 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.37; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol, 80:20, 1 mLmin^{-1}, λ = 254 nm): t_R= 10.0 and 16.7 min; $[\alpha]_D^{30}$ = 0.0 (*c* 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, CDCl3): δ_H = 7.37-7.19 (5H, m, C_6H_5), 4.75-4.67 (1H, m, NC*H*), 4.26-4.08 (3H, m, CHCH_2O, C*H*OH), 3.75 (1H, dq, *J* = 2.9 and 7.0 Hz, COC*H*CH_3), 3.25 (1H, dd, *J* = 3.3 and 13.4 Hz, CH*H*Ph), 2.91 (1H, d, *J* = 2.7 Hz, O*H*), 2.79 (1H, dd, *J* = 9.4 and 13.4 Hz, C*H*HPh), 1.27 (3H, d, *J* = 7.1 Hz, COCHCH_3), 1.21 (3H, d, *J* = 6.4 Hz, CHOHCH_3).

General procedure for the enzymatic resolution of *rac-syn*-5.8

In a typical experiment racemic alcohol ***rac-syn*-5.8** (0.050 g, 0.18 mmol) and *Candida antarctica* lipase type B (0.006 g, 30 mg_{enzyme}/$mmol_{substrate}$) in organic solvent (vinyl acetate, *n*-hexane, toluene, dichloromethane or diisopropyl ether, 0.8 ml) were stirred with vinyl acetate (33 μL, 0.36 mmol) at 40 °C. After 24 hours, the reaction was stopped by filtering off the enzyme. Conversions and enantiomeric excesses obtained in the different solvents are reported in Table 5.8. Ester **(4*S*,2'*S*,3'*R*)-5.9** (0.025 g, 43% yield for the reaction performed in *n*-hexane) was separated from unreacted alcohol **(4*R*,2'*R*,3'*S*)-5.8** (0.019 g, 39% yield for the reaction performed in *n*-hexane) by flash chromatography (SiO_2, *n*-hexane/EtOAc 60:40). Spectroscopic, chromatographic and physical-chemical data were in accordance with those previously reported (*vide infra*).

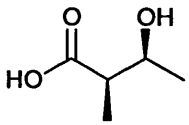
Hydrolysis of alcohol (4*R*,2'*R*,3'*S*)-5.8[14]

To a solution of alcohol **(4*R*,2'*R*,3'*S*)-5.8** (0.332 g, 1.20 mmol) in THF/H_2O (5 mL, 80:20) at 0 °C, 30% aqueous hydrogen peroxide (400 μL) was added slowly. LiOH (0.038 g, 1.58 mmol) in water (2 mL) was then added and the mixture was allowed to stir at 0 °C for 2 hours. The reaction was quenched with a solution of Na_2SO_3 in water (1.5 M, 3 mL) and stirred until peroxides were no longer detectable. The bulk of THF

was removed *in vacuo* and the resulting mixture (pH 12-13) was extracted with DCM (3 × 5 mL) to remove the chiral auxiliary. The aqueous layer was cooled in an ice bath and acidified to pH 1 by addition of 6 M HCl. The resulting cloudy solution containing the β-hydroxy acid **(2*R*,3*S*)-5.14** was then extracted with EtOAc (4 × 5 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil, which was dissolved in 5% aqueous $NaHCO_3$ (2 mL). This solution was extracted with DCM, acidified with 6 M HCl and extracted with EtOAc as before. The combined ethyl acetate exracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford β-hydroxy acid **(2*R*,3*S*)-5.14** as a yellow oil (0.085 g, 60%).

The combined DCM extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford oxazolidinone **(*R*)-5.10** (commercially available compound) as a white solid (0.180 g, 85%).

(2*R*,3*S*)-3-Hydroxy-2-methylbutanoic acid (2*R*,3*S*)-5.14

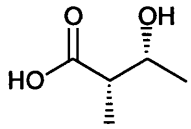


Yellow oil (0.085 g, 60%); $[\alpha]_D^{30}$ = +6.9 (*c* 1.02, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ_H = 5.8 (2H, br s, 2 × O*H*), 4.15-4.05 (1H, dq, app. m, C*H*OH), 2.59 (1H, dq, *J* = 3.7 and 7.2 Hz, COC*H*CH_3), 1.23 (3H, d, *J* = 3.9 Hz, COCHC*H*$_3$), 1.22 (3H, d, *J* = 3.1 Hz, CHOHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C= 180.5 (*C*OOH), 67.9 (CO*C*HCH_3), 45.2 (*C*HOH), 19.7 (CHOH*C*H_3), 10.9 (COCH*C*H_3). ^1H and ^{13}C NMR data were in accordance with those reported in literature.[10]

Hydrolysis of ester (4*S*,2'*S*,3'*R*)-5.9[14]

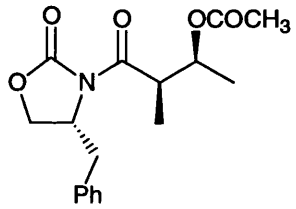
Hydrolysis of ester **(4*S*,2'*S*,3'*R*)-5.9** was carried out following the same procedure above described for hydrolysis of alcohol **(4*R*,2'*R*,3'*S*)-5.8**. Ester **(4*S*,2'*S*,3'*R*)-5.9** (0.160 g, 0.50 mmol) was hydrolysed with LiOH (0.016 g, 0.66 mmol) in H_2O_2 to afford 4-benzyl-2-oxazolidinone **(*S*)-5.10** (commercially available compound) as a white solid (0.074 g, 84%) and β-hydroxy acid **(2*S*,3*R*)-5.14** (0.032 g, 55%) as a yellow oil.

(2*S*,3*R*)-3-Hydroxy-2-methylbutanoic acid (2*S*,3*R*)-5.14



Yellow oil (0.032 g, 55%); $[\alpha]_D^{30}$ = -6.8 (*c* 1.02, $CHCl_3$); ^1H and ^{13}C NMR data were in accordance with those reported in literature.[10]

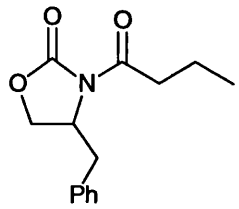
Preparation of (4*R*,2'*R*,3'*S*)-*N*-(3-acetoxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.9[13]



Acetic acid (20 μL, 0.35 mmol) and DMAP (4-dimethylaminopyridine) (0.035 g, 0.28 mmol) were added to a solution of alcohol **(4*R*,2'*R*,3'*S*)-5.8** (0.296 g, 1.07 mmol) in anhydrous dichloromethane (5 mL). The solution was stirred, cooled in an ice bath to 0 °C and DCC (dicyclohexylcarbodiimide) (0.080 g, 0.39 mmol) was added over a period of 5 min. After a further 5 min at 0 °C, the ice bath was removed and the mixture was allowed to react for 3 hours at room temperature. Precipitated dicyclohexylurea was removed by filtration and the filtrate was washed with 5 M HCl (2 × 3 mL) and sat. $NaHCO_3$ solution (2 × 3 mL). During this procedure some additional dicyclohexylurea was precipitated, which was removed by filtration of both layers to facilitate their separation. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification *via* flash chromatography (*n*-hexane/EtOAc, 60:40) afforded acylated aldol adduct **(4*R*,2'*R*,3'*S*)-5.9** as yellow needles (0.220 g, 65%); mp 81-82 °C; R_f (*n*-hexane/EtOAc, 60:40) = 0.65; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 80:20, 1 $mLmin^{-1}$, λ= 254 nm): t_R = 7.6 min; $[\alpha]_D^{30}$ = -75.5 (*c* 0.96, $CHCl_3$); IR ($CHCl_3$): ν = 3520, 2980, 1780, 1735, 1700, 1450, 1380, 1235, 1110 cm^{-1}; ^1H NMR (300 MHz, CDCl3): δ_H = 7.36-7.19 (5H, m, C_6H_5), 5.30 (1H, dq, *J* = 4.3 and 6.4 Hz, $CHOCOCH_3$), 4.64-4.56 (1H, m, NC*H*), 4.28-4.08 (2H, m, CH_2O), 3.95 (1H, dq, *J* = 4.3 and 6.8 Hz, $COCHCH_3$), 3.27 (1H, dd, *J* = 3.2 and 13.3

Hz, CH*H*Ph), 2.76 (1H, dd, *J* = 9.7 and 13.3 Hz, C*H*HPh), 2.05 (3H, s, OCOCH_3), 1.28 (3H, d, *J* = 6.4 Hz, COCHCH_3), 1.23 (3H, d, *J* = 6.9 Hz, AcOCHCH_3); ^{13}C NMR (75.5 MHz, CDCl3): δ_C = 172.2 (*C*O), 168.8 (O*C*OCH$_3$), 151.7 (O*C*ON), 135.8, 129.9 (2C), 129.4 (2C), 127.8 (aromatic C), 70.8 (*C*HOCOCH$_3$), 66.8 (*C*H$_2$O), 56.2 (N*C*H), 42.8 (CO*C*HCH$_3$), 38.4 (*C*H$_2$Ph), 21.6 (OCO*C*H$_3$), 18.5 (AcOCH*C*H$_3$), 11.3 (COCH*C*H$_3$); MS (FAB+): *m/z* 320 (MH$^+$, 88%); Anal. calcd. for C_{17} $H_{21}NO_5$: C 63.93%, H 6.62%, N 4.38%; found: C 64.4%, H 6.8%, N 4.0%.

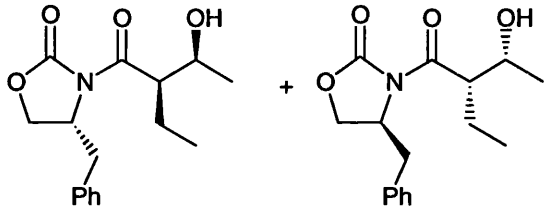
Preparation of (*R/S*)-(+/-)-3-butyryl-4-benzyl-2-oxazolidinone *rac*-5.15[14]



A solution of *n*-BuLi in *n*-hexane (2.5 M, 5.3 mL, 13.2 mmol) was added dropwise, over a period of 10 min, to a stirred solution of ***rac*-5.10** (2.0 g, 13.2 mmol) in anhydrous THF (60 mL) at -78 °C under an atmosphere of nitrogen. The mixture was stirred at this temperature for 15 min and *n*-PrCOCl (1.6 mL, 15.4 mmol) was then added dropwise over 5 min. After 30 min the reaction quenched with sat. NH_4Cl solution (40 mL) and allowed to warm to room temperature over 2 hour period. The mixture was extracted with EtOAc (3 x 40 mL); the organic extracts were combined and washed with brine (40 mL). The dried solution ($MgSO_4$) was concentrated *in vacuo* and the residue was purified by recrystallisation (*n*-hexane) to give ***rac*-5.15** as a colourless crystalline solid (2.5 g, 76%); mp 31-33°C; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 93:7, 1 mLmin^{-1}, λ= 254 nm): t_R=15.4 and 15.8 min; IR (CH_2Cl_2): ν = 2965, 1780, 1700, 1385, 1355, 1215,1090 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.20 (5H, m, C$_6$*H*$_5$), 4.71-4.63 (1H, m, C*H*N), 4.23-4.14 (2H, m, C*H*$_2$O), 3.30 (1H, dd, *J* = 3.2 and 13.3 Hz, CH*H*Ph), 2.89-2.82 (2H, m, C*H*$_2$CH$_2$CH$_3$), 2.76 (1H, dd, *J* = 9.7 and 13.3 Hz, C*H*HPh), 1.79-1.62 (2H, m, C*H*$_2$CH$_3$), 1.01 (3H, t, *J* = 7.4 Hz, C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C= 173.6 (*C*O), 153.8 (O*C*ON), 135.7, 129.8 (2C), 129.3 (2C), 127.7 (aromatic C), 66.5 (*C*H$_2$O), 55.5 (*C*HN), 38.3 (*C*H$_2$Ph), 37.7 (*C*H$_2$CH$_2$CH$_3$), 18.1 (*C*H$_2$CH$_3$), 14.1 (*C*H$_3$); MS (EI+, 70 eV): *m/z* 247 (M$^+$, 10%), 177 (9), 156 (15), 143 (31), 129 (55), 119 (14), 101 (8), 91 (16), 71 (100), 55 (17), 43 (45); Anal. calcd. for $C_{14}H_{17}NO_3$: C 68.00%, H 6.93%, N 5.66%; found: C

67.9%, H 7.1%, N 5.3%. ^{1}H and ^{13}C NMR data were in accordance with those reported in literature.[17]

Preparation of (4*R*,2'*R*,3'*S*)- and (4*S*,2'*S*,3'*R*)-*N*-(3-hydroxy-2-ethylbutanoyl)-4-benzyl-2-oxazolidinone *rac-syn*-5.16[12]



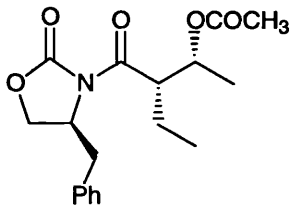
$TiCl_4$ (1M solution in DCM, 6.5 mL, 6.45 mmol) was added to a solution of *N*-butyryl-2-oxazolidinone ***rac*-5.15** (1.5 g, 6.15 mmol) in dichloromethane (50 mL) at 0 °C under an atmosphere of nitrogen. The mixture was stirred for 5 min and (-)-sparteine (1.4 mL, 6.15 mmol) was then added dropwise slowly. After complete addition and 20 min at 0 °C, the mixture was cooled to -78 °C and *N*-methyl-2-pyrrolidinone (600 μL, 6.15 mmol) was added. The reaction mixture was stirred for 10 min, followed by addition of freshly distilled acetaldehyde (1.3 mL, 30.7 mmol) dropwise. The mixture was allowed to react for 1 h at -78 °C, gradually warmed to 0 °C, and stirred for 1 h. The reaction was quenched with half-saturated NH_4Cl (30 mL) and warmed to 25 °C. The layers were separated and the aqueous layer was extracted twice with DCM (2 × 30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification *via* flash chromatography (SiO_2, *n*-hexane/EtOAc, 50:50) afforded ***rac-syn*-5.16** (1.0 g, 59%, 95% d.e.) as a cream solid; mp 72-74 °C (Lit.[10] 74-76 °C); R_f (*n*-hexane/EtOAc, 50:50) = 0.55; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 90:10, 1 mLmin^{-1}, λ= 254 nm: t_R=14.6 and 20.4 min; $[\alpha]_D^{30}$ = 0.0 (*c* 0.99, $CHCl_3$); IR (CH_2Cl_2): ν = 3570, 2975, 1775, 1690, 1385, 1355, 1215, 1105 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37- 7.22 (5H, m, C_6H_5), 4.77-4.70 (1H, m, NC*H*), 4.22-4.09 (3H, m, CH_2O, C*H*OH), 4.03-3.97 (1H, m, C*H*CH_2CH_3), 3.36 (1H, dd, *J* = 3.2 and 13.2 Hz, CH*H*Ph), 2.71 (1H, dd, *J* = 10.1 and 13.2 Hz, C*H*HPh), 2.53 (1H, s, O*H*), 1.91-1.83 (1H, m, CH*H*CH_3), 1.74-1.66 (1H, m, C*H*HCH_3), 1.23 (3H, d, *J* = 6.4 Hz, $CHCH_3$), 0.98 (3H, t, *J* = 7.4 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 175.9 (*C*O), 154.3 (O*C*ON), 135.6, 129.7 (2C), 129.3 (2C), 127.7 (aromatic C), 69.0 (*C*HOH), 66.4 (CH_2O), 55.9 (*C*HN), 50.4 ($CHCH_2CH_3$), 38.4 (CH_2Ph), 21.1 (CH_2CH_3), 19.9 ($CHCH_3$), 12.3 (CH_2CH_3); MS (EI+, 70 eV): *m/z* 291 (M^{+}, 23%), 273 (27), 258 (53), 247 (50), 115 (25), 97 (21), 86

(65), 71 (100), 43 (56); Anal. calcd. for $C_{16}H_{21}NO_4$: C 65.96%, H 7.27%, N 4.81%; found: C 65.6%, H 7.2%, N 4.5%.

Enzymatic resolution of *rac-syn*-5.16

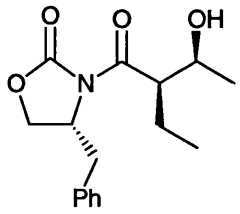
Racemic alcohol ***rac-syn*-5.16** (0.932 g, 3.20 mmol) and *Candida antarctica* lipase type B (0.324 g, 100 $mg_{enzyme}/mmol_{substrate}$) in *n*-hexane (16 mL) were stirred with vinyl acetate (590 μL, 6.40 mmol) at 40 °C. After 20 hours, the reaction was stopped by filtering off the enzyme. Ester **(4*S*,2'*S*,3'*R*)-5.17** (0.373 g, 35%) was separated from unreacted alcohol **(4*R*,2'*R*,3'*S*)-5.16** (0.265 g, 30%) by flash chromatography (SiO_2, *n*-hexane/EtOAc 50:50) and recrystallised from *n*-hexane/EtOAc.

(4*S*,2'*S*,3'*R*)-*N*-(3-Acetoxy-2-ethylbutanoyl)-4-benzyl-2-oxazolidinone (4*S*,2'*S*,3'*R*)-5.17



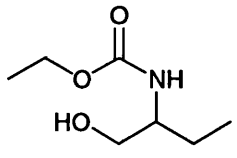
Ester **(4*S*,2'*S*,3'*R*)-5.17** was isolated as a cream solid; mp 61-63 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.85; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 90:10, 1 $mLmin^{-1}$, λ= 254 nm): t_R=11.5 min; $[\alpha]_D^{30}$ = +57.1 (*c* 1.05, $CHCl_3$); IR (CH_2Cl_2): ν = 2965, 1780, 1730, 1695, 1380, 1235, 1110 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.36-7.21 (5H, m, C_6H_5), 5.30-5.22 (1H, m, $CHCH_3$), 4.72-4.64 (1H, m, NC*H*), 4.24-4.10 (2H, m, CH_2O), 4.04-3.98 (1H, m, $CHCH_2CH_3$), 3.32 (1H, dd, *J* = 3.2 and 13.2 Hz, CH*H*Ph), 2.73 (1H, dd, *J* = 9.9 and 13.2 Hz, C*H*HPh), 2.01 (3H, s, $OCOCH_3$), 1.91-1.83 (1H, m, $CHHCH_3$), 1.74-1.66 (1H, m, $CHHCH_3$), 1.27 (3H, d, *J* = 6.7 Hz, $CHCH_3$), 0.95 (3H, t, *J* = 7.4 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 173.3 (*C*O), 170.4 O*C*OCH3), 153.4 (O*C*ON), 135.3, 129.4 (2C), 128.9 (2C), 127.3 (aromatic C), 70.1 ($CHCH_3$), 66.1 (CH_2O), 55.7 (*C*HN), 48.6 ($CHCH_2CH_3$), 38.1 (CH_2Ph), 21.1 (OCO*C*H3), 20.0 (CH_2CH_3), 17.7 ($CHCH_3$), 11.7 (CH_2CH_3); MS (EI+, 70 eV): *m/z* 333 (M^{+}, 30%), 258 (20), 157 (32), 97 (100), 69 (29), 43 (50); Anal. calcd. for $C_{18}H_{23}NO_5$: C 64.85%, H 6.95%, N 4.20%; found: C 64.7%, H 7.0%, N 4.0%.

(4*R*,2'*R*,3'*S*)-*N*-(3-Hydroxy-2-ethylbutanoyl)-4-benzyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.16



Alcohol **(4*R*,2'*R*,3'*S*)-5.16** was isolated as a cream solid; mp 73-74 °C (Lit.[7] 74-76 °C); R_f (*n*-hexane/EtOAc, 50:50) = 0.55; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 90:10, 1 mLmin^{-1}, λ= 254 nm): t_R=14.6 min; $[\alpha]_D^{30}$ = -15.8 (*c* 1.2, $CHCl_3$); IR (CH_2Cl_2): ν = 3590, 2980, 1780, 1690, 1385, 1350, 1215, 1110 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 7.37-7.22 (5H, m, C_6H_5), 4.78-4.70 (1H, m, NC*H*), 4.22-4.07 (3H, m, CH_2O, C*H*OH), 4.03-3.97 (1H, m, C*H*CH_2CH_3), 3.36 (1H, dd, *J* = 3.2 and 13.2 Hz, CH*H*Ph), 2.70 (1H, dd, *J* = 10.0 and 13.2 Hz, C*H*HPh), 2.54 (1H, s, O*H*), 1.91-1.83 (1H, m, CH*H*CH_3), 1.74-1.66 (1H, m, C*H*HCH_3), 1.24 (3H, d, *J* = 7.0 Hz, $CHCH_3$), 0.98 (3H, t, *J* = 7.4 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C= 175.5 (*C*O), 153.9 (O*C*ON), 135.2, 129.4 (2C), 129.0 (2C), 127.4 (aromatic C), 68.6 (*C*HOH), 66.0 (CH_2O), 55.6 (*C*HN), 50.1 (*C*HCH_2CH_3), 38.1 (CH_2Ph), 20.7 (CH_2CH_3), 19.5 ($CHCH_3$), 11.9 (CH_2CH_3); MS (EI+, 70 eV): *m/z* 291 (M^{+}, 11%), 158 (26), 128 (27), 99 (100), 86 (67), 71 (77), 53 (33), 43 (90); Anal. calcd. for $C_{16}H_{21}NO_4$: C 65.96%, H 7.27%, N 4.81%; found: C 65.6%, H 7.2%, N 4.6%. See Appendices for crystal structure.

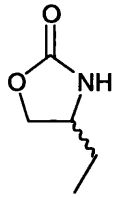
Preparation of *N*-[*sec*-(1-hydroxybutyl)]carbamic acid ethyl ester 5.19[2]



2-Amino-1-butanol ***rac*-5.18** (12.6 mL, 133 mmol) was dissolved in a solution of Na_2CO_3 (50.0 g, 472 mmol) in H_2O (100 mL). The mixture was cooled to 5 °C and EtO_2CCl (14.0 mL, 134 mmol) was added. After stirring at room temperature for another 1.5 hours, the mixture was filtered and extracted with EtOAc (70 x 4 mL).

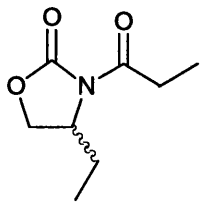
The combined organic extracts were washed with H_2O (30 mL) and brine (30 mL) and dried over $MgSO_4$. Removal of the solvent gave ***rac*-5.19** as a white solid; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 5.03 (1H, br s, N*H*), 4.10 (2H, q, *J* = 7.1 Hz, COOC*H*$_2$CH$_3$), 3.69-3.54 (3H, m, C*H*$_2$OH, C*H*N), 3.03 (1H, s, O*H*), 1.61-1.41 (2H, m, CHC*H*$_2$CH$_3$), 1.24 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$), 0.95 (3H, t, *J* = 7.4 Hz, CHCH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 157.6 (N*C*O), 65.4 (*C*H$_2$OH), 61.3 (COO*C*H$_2$CH$_3$), 54.9 (*C*HN), 24.7 (CH*C*H$_2$CH$_3$), 14.9 (OCH$_2$*C*H$_3$), 10.8 (CHCH$_2$*C*H$_3$).

Preparation of (*R*/*S*)-(+/-)-4-ethyl-2-oxazolidinone *rac*-5.20[2]



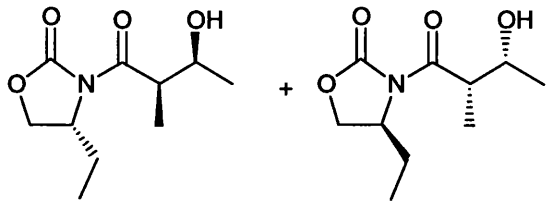
Powdered K_2CO_3 (0.093 g, 0.14 mmol) was added to carbamic acid ethyl ester ***rac*-5.19** and the mixture was heated to 125 °C with magnetic stirring under vacuum until the gas evolution stopped (approximately 2 hours). The mixture was dissolved H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$), run through silica to eliminate K_2CO_3 in excess, and concentrated *in vacuo* to afford ***rac*-5.20** as a yellow oil (10.6 g, 70% overall yield from ***rac*-5.18**); R_f (*n*-hexane/EtOAc, 66:34) = 0.11; IR (film): ν = 3290, 2965, 2935, 1745, 1405, 1235, 1050 cm^{-1}; $[\alpha]_D^{30}$= 0.0 (*c* 0.63, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.0 (1H, br s, N*H*), 4.48 (1H, app. t, *J* = 8.5 Hz, CH*H*O), 4.02 (1H, dd, *J* = 6.0 and 8.4 Hz, C*H*HO), 3.84-3.78 (1H, m, C*H*N), 1.65-1.55 (2H, m, C*H*$_2$CH$_3$), 0.95 (3H, t, *J* = 7.6 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 160.9 (O*C*ON), 70.4 (*C*H$_2$O), 54.2 (*C*HN), 28.5 (*C*H$_2$CH$_3$), 9.6 (*C*H$_3$). ^1H NMR data were in accordance with those reported in literature.[18]

Preparation of (*R*/*S*)-(+/-)-3-propionyl-4-ethyl-2-oxazolidinone *rac*-5.21[14]



A solution of *n*-BuLi in *n*-hexane (1.6 M, 5.0 mL, 7.96 mmol) was added dropwise, over a period of 10 min, to a stirred solution of ***rac*-5.20** (916 mg, 7.96 mmol) in anhydrous THF (30 mL) at -78 °C under an atmosphere of nitrogen. The mixture was stirred at this temperature for 15 min and EtCOCl (690 μL, 7.96 mmol) was then added dropwise over 5 min. After 30 min the reaction quenched with sat. NH_4Cl solution (20 mL) and allowed to warm to room temperature over 2 hour period. The mixture was extracted with EtOAc (3 x 20 mL); the organic extracts were combined and washed with brine (20 mL). The dried solution ($MgSO_4$) was concentrated *in vacuo* and the residue was purified *via* flash chromatography (SiO_2, *n*-hexane/EtOAc 66:34) to give ***rac*-5.21** as a colourless oil (0.875 g, 64%); R_f (*n*-hexane/EtOAc, 66:34) = 0.61; HPLC: (Chiralcel® AD column, *n*-hexane/isopropanol 90:10, 1 $mLmin^{-1}$, λ = 254): t_R = 8.3 and 10.2 min; IR (film): 2970, 2940, 2885, 1780, 1705, 1390, 1375, 1250, 1210, 1070, 1020 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 4.45-4.36 (2H, m, OCH_2), 4.15-4.11 (1H, m, CHN), 2.96-2.86 (2H, m, $COCH_2CH_3$), 1.87-1.69 (2H, m, $CHCH_2CH_3$), 1.16 (3H, t, *J* = 7.3 Hz, $COCH_2CH_3$), 0.92 (3H, t, *J* = 7.5 Hz, $CHCH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 173.9 (CO), 153.9 (OCON), 66.8 (CH_2O), 55.0 (CHN), 29.1 ($COCH_2CH_3$), 25.1 ($CHCH_2CH_3$), 8.3 ($CHCH_2CH_3$), 8.1 ($COCH_2CH_3$); MS (EI+, 70 eV): *m/z* 171 (M^{+}, 15%), 142 (16), 116 (18), 86 (8), 57 (100), 28 (52); Anal. calcd. for $C_8H_{13}NO_3$: C 56.13%, H 7.65%, N 8.18%; found: C 56.0%, H 7.6%, N 7.9%.

Preparation of (4*R*,2'*R*,3'*S*)- and (4*S*,2'*S*,3'*R*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-ethyl-2-oxazolidinone *rac-syn*-5.22[12]



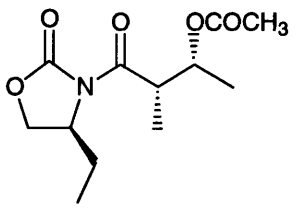
$TiCl_4$ (1M solution in DCM, 2.6 mL, 2.58 mmol) was added to a solution of *N*-propionyl-2-oxazolidinone ***rac*-5.21** (0.421 g, 2.46 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was stirred for 5 min and (-)-sparteine (560 μL, 2.46 mmol) was then added dropwise. After complete addition and 20 min at 0 °C, the mixture was cooled to -78 °C and *N*-methyl-2-pyrrolidinone (240 μL, 2.46 mmol) was added. The

reaction mixture was stirred for 10 min, followed by addition of freshly distilled acetaldehyde (0.5 mL, 12.3 mmol) dropwise. The mixture was allowed to react for 1 h at -78 °C, gradually warmed to 0 °C, and stirred for 1 h. The reaction was quenched with half-saturated NH_4Cl (20 mL) and warmed to 25 °C. The layers were separated and the aqueous layer was extracted twice with DCM (2 × 20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification *via* flash chromatography (SiO_2, *n*-hexane/EtOAc, 40:60) afforded ***rac-syn*-5.22** (0.293 g, 55%, >99% d.e.) as a colourless oil; R_f (*n*-hexane/EtOAc, 40:60) = 0.53; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 80:20, 1 mLmin^{-1}, λ = 254): t_R= 7.3 and 11.3 min; $[\alpha]_D^{30}$ = 0.0 (*c* 0.63, CCl_3); IR (film): ν = 3465, 2970, 2940, 2885, 1775, 1690, 1385, 1230, 1205 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 4.50-4.45 (1H, m, C*H*N); 4.40 (1H, app. t, *J* = 8.2, OCH*H*), 4.15-4.11 (2H, m, J = 2.7 Hz, C*H*OH, OC*H*H), 3.72 (1H, dq, *J* = 2.9 and 7.0 Hz, COC*H*CH_3), 3.02 (1H, s, O*H*), 1.83-1.72 (2H, m, C$H_2$$CH_3$), 1.24 (3H, d, *J* = 7.0 Hz, COCHCH_3), 1.19 (3H, d, *J* = 6.4 Hz, CHOHCH_3), 0.93 (3H, t, *J* = 7.5 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 177.5 (C*C*O), 153.4 (O*C*ON), 67.5 (*C*HOH), 66.7 (*C*H_2O), 54.9 (*C*HN), 43.0 (CO*C*HCH_3), 24.9 (*C*H_2CH_3), 19.5 (CHOH*C*H_3), 10.6 (COCH*C*H_3), 8.0 (CH_2*C*H_3); MS (CI+) : *m/z* 216 (M^+, 80%), 198 (100), 171 (35), 156 (14), 142 (18), 116 (64), 83 (20) ; Anal. calcd. for $C_{10}H_{17}NO_4$: C 55.80%, H 7.96%, N 6.51%; found: C 55.4%, H 8.0%, N 6.3%.

Enzymatic resolution of *rac-syn*-5.22

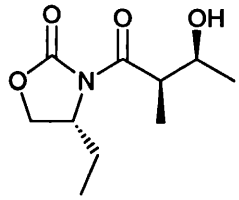
Racemic alcohol ***rac-syn*-5.22** (0.250 g, 1.16 mmol) and *Candida antarctica* lipase type B (0.116 g, 100 mg_{enzyme}/$mmol_{substrate}$) in *n*-hexane (5 mL) were stirred with vinyl acetate (214 μL, 2.32 mmol) at 40 °C. After 20 hours, the reaction was stopped by filtering off the enzyme. Ester **(4*S*,2'*S*,3'*R*)-5.23** (0.098 g, 33%) was separated from unreacted alcohol **(4*R*,2'*R*,3'*S*)-5.22** (0.090 g, 36%) by flash chromatography (SiO_2, *n*-hexane/EtOAc 50:50).

(4*S*,2'*S*,3'*R*)-*N*-(3-Acetoxy-2-methylbutanoyl)-4-ethyl-2-oxazolidinone (4*S*,2'*S*,3'*R*)-5.23



Ester **(4*S*,2'*S*,3'*R*)-5.23** was isolated as a yellow oil; HPLC (Chiralcel® OD column, *n*-exane/isopropanol 80:20, 1 mLmin^{-1}, λ = 254): t_R= 6.0; $[\alpha]_D^{30}$ = -86.6 (*c* 0.30, $CHCl_3$); IR (film): ν = 2985, 2940, 1780, 1735, 1385, 1370, 1700, 1240, 1210 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 5.27 (1H, dq, *J* = 3.2 and 6.4 Hz, C*H*OCOCH$_3$), 4.44-4.35 (2H, m, C*H*N, OCH*H*), 4.13 (1H, dd, *J* = 1.8 and 8.0 Hz, OC*H*H), 3.96 (1H, dq, *J* = 3.4 and 6.9 Hz, COC*H*CH$_3$), 2.02 (3H, s, OCOC*H*$_3$), 1.82-1.72 (2H, m, C*H*$_2$CH$_3$), 1.26 (3H, d, *J* = 6.4 Hz, COCHC*H*$_3$), 1.19 (3H, d, *J* = 6.9 Hz, AcOCHC*H*$_3$), 0.92 (3H, t, *J* = 7.5 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 174.0 (C*C*O), 170.6 (O*C*OCH$_3$), 153.8 (O*C*ON), 70.3 (*C*HOCOCH$_3$), 66.8 (*C*H$_2$O), 55.6 (*C*HN), 42.1 (CO*C*HCH$_3$), 25.1 (*C*H$_2$CH$_3$), 21.1 (OCO*C*H$_3$), 18.1 (AcOCH*C*H$_3$), 11.0 (COCH*C*H$_3$), 8.2 (CH$_2$*C*H$_3$); MS (CI+) : *m/z* 258 (MH$^+$, 55%), 198 (100), 171 (12), 143 (21), 83 (40) ; Anal. calcd. for $C_{12}H_{19}NO_5$: C 56.02%, H 7.44%, N 5.44%; found C 55.6%, H 7.5%, N 5.4%.

(4*R*,2'*R*,3'*S*)-*N*-(3-Hydroxy-2-methylbutanoyl)-4-ethyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.22



Unreacted alcohol **(4*R*,2'*R*,3'*S*)-5.22** was obtained as a colourless oil; R_f (*n*-hexane/EtOAc, 40:60) = 0.53; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 80:20, 1 mLmin^{-1}, λ = 254): t_R = 7.3 min; $[\alpha]_D^{30}$ = -40.0 (*c* 0.35, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 4.50-4.45 (1H, m, C*H*N), 4.40 (1H, app. t, *J* = 8.5 Hz, OCH*H*), 4.17-4.10 (2H, m, OC*H*H, C*H*OH), 3.71 (1H, dq, *J* = 3.5 and 7.1 Hz, COC*H*CH$_3$), 2.98 (1H, s, O*H*), 1.82-1.72 (2H, m, C*H*$_2$CH$_3$), 1.24 (3H, d, *J* = 7.0 Hz, COCHC*H*$_3$), 1.19 (3H, d, *J* = 6.4 Hz, CHOHC*H*$_3$), 0.93 (3H, t, *J* = 7.5 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 177.9 (C*C*O), 153.8 (O*C*ON), 67.8 (*C*HOH), 67.1 (*C*H$_2$O), 55.3 (*C*HN), 43.4 (CO*C*HCH$_3$), 25.3 (*C*H$_2$CH$_3$), 19.9 (CHOH*C*H$_3$), 11.0 (COCH*C*H$_3$), 8.4 (CH$_2$*C*H$_3$); MS (CI+) : *m/z* 216 (MH$^+$, 100%), 198 (81), 171 (16), 142 (11), 116 (23), 83 (11) ; Anal. calcd. for $C_{10}H_{17}NO_4$: C 55.80%, H 7.96%, N 6.51% ; found C 55.6%, H 7.9%, N 6.6%.

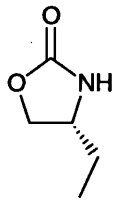
Hydrolysis of alcohol (4*R*,2'*R*,3'*S*)-5.22[14]

To a solution of alcohol **(4*R*,2'*R*,3'*S*)-5.22** (0.080 g, 0.37 mmol) in THF/H_2O (2 mL, 80:20) at 0 °C, 30% aqueous hydrogen peroxide (15 μL) was added slowly. LiOH (0.012 g, 0.50 mmol) in water (1 mL) was then added and the mixture was allowed to stir at 0 °C for 2 hours. The reaction was quenched with a solution of Na_2SO_3 in water (1.5 M, 1 mL) and stirred until peroxides were no longer detectable. The bulk of THF was removed *in vacuo* and the resulting mixture (pH 12-13) was extracted with DCM (3 × 1.5 mL) to remove the chiral auxiliary.

The aqueous layer was cooled in an ice bath and acidified to pH 1 by addition of 6 M HCl. The resulting cloudy solution containing the β-hydroxy acid **(2*R*,3*S*)-5.14** was then extracted with EtOAc (4 × 2 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil, which was dissolved in 5% aqueous $NaHCO_3$ (0.5 mL). This solution was extracted with DCM, acidified with 6 M HCl and extracted with EtOAc as before. The combined ethyl acetate extracts were finally dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford β-hydroxy acid **(2*R*,3*S*)-5.14** as a yellow oil (0.023 g, 53%). Spectroscopic data and specific rotation were in accordance with those previously reported.

The combined DCM extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford oxazolidinone **(*R*)-5.20** as a colourless oil (0.036 g, 82%).

(*R*)-(+)-4-Ethyl-2-oxazolidinone (*R*)-5.20

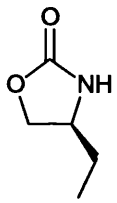


Yellow oil; $[\alpha]_D^{30}$ = +5.5 (*c* 0.63, $CHCl_3$), (Lit.[15] +5.8 (c 1.02, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ_H = 6.52 (1H, br s, N*H*), 4.48 (1H, app. t, *J* = 8.5 Hz, CH*H*O), 4.03 (1H, dd, *J* = 6.0 and 8.5 Hz, C*H*HO), 3.86-3.77 (1H, m, C*H*N), 1.66-1.55 (2H, m, C$H_2$$CH_3$),0.92 (3H, t, *J* = 7.5 Hz, CH_2CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 160.3 (N*C*O), 70.0 (*C*H_2O), 53.8 (*C*HN), 28.2 (*C*H_2CH_3), 9.3 (*C*H_3).

Hydrolysis of ester (4*S*,2'*S*,3'*R*)-5.23 [14]

Hydrolysis of ester **(4*S*,2'*S*,3'*R*)-5.23** was carried out following the same procedure above described for hydrolysis of alcohol **(4*R*,2'*R*,3'*S*)-5.22**. Ester **(4*S*,2'*S*,3'*R*)-5.9** (0.095 g, 0.37 mmol) was hydrolysed with LiOH (0.012 g, 0.50 mmol) in H_2O_2 to afford 4-ethyl-2-oxazolidinone **(*S*)-5.20** as a colourless oil (0.037 g, 87%) and β-hydroxy acid **(2*S*,3*R*)-5.14** (0.022 g, 51%) as a yellow oil.
Spectroscopic data and specific rotation for β-hydroxy acid **(2*S*,3*R*)-5.14** were in accordance with those previously reported.

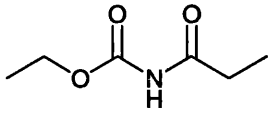
(*S*)-(-)-4-Ethyl-2-oxazolidinone (*S*)-5.20



Yellow oil; $[\alpha]_D^{30}$ = -5.3 (*c* 0.60, $CHCl_3$). ^1H and ^{13}C NMR data were in accordance with those above reported for the (*R*)-enantiomer **(*R*)-5.20.**

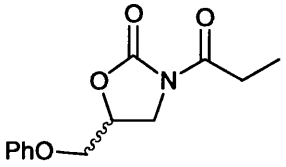
7.5 Experimental for Chapter 6

Preparation of *N*-propionyl-carbamic acid ethyl ester 6.30[19]



A mixture of carbamic acid ethyl ester (urethane) (2.9 g, 33.3 mmol) and propionyl chloride (32.0 mL, 36.8 mmol) was refluxed until HCl evolution ceased (approximately 2 hours). The brown oil was cooled to room temperature and crystallised from petroleum ether to give **6.30** (2.8 g, 58%) as a white solid; mp 77-79 °C (Lit.[16] 81-82 °C); R_f (DCM/*n*-hexane/isopropanol, 60:35:5) = 0.65; IR (KBr): ν = 3260, 3190, 2975, 1760, 1515, 1240 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.95 (1H, br s, N*H*), 4.21 (2H, q, *J* = 7.1 Hz, $COOCH_2CH_3$), 2.78 (2H, q, *J* = 7.4 Hz, $NCOCH_2CH_3$), 1.30 (3H, t, *J* = 7.1 Hz, $COOCH_2CH_3$), 1.16 (3H, t, *J* = 7.4 Hz, $NCOCH_2CH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 176.1 (CH_2CON), 152.9 (N*C*OO), 62.6 ($COOCH_2CH_3$), 29.9 ($NCOCH_2CH_3$), 14.6 ($COOCH_2CH_3$), 8.6 ($NCOCH_2CH_3$); MS (FAB+): *m/z* 146 (MH^+, 39%); Anal. calcd. for $C_6H_{11}NO_3$: C 49.65%, H 7.64%, N 9.65%; found: C 49.3%, H 7.5%, N 9.8%.

Preparation of (*R*/*S*)-(+/-)-*N*-propionyl-5-phenoxymethyl-2-oxazolidinone 6.31[20]



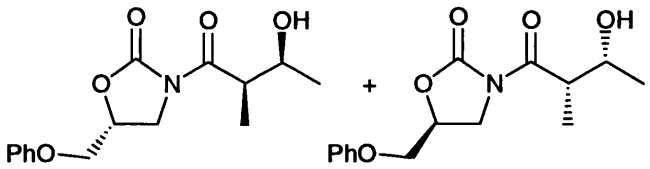
A solution of *N*-propionyl-carbamic acid ethyl ester **6.30** (0.534 g, 3.67 mmol) and 1,2-epoxy-3-phenoxypropane **6.25** (0.555 g, 3.70 mmol) in dry toluene (1 mL) was heated to 70 °C. DABCO (1,4-diazabicyclo[2.2.2.]octane) (0.010 g, 0.09 mmol, 2.5% mol) was added and the reaction mixture was refluxed for 3 hours under vacuum. The crude white solid was treated with boiling cyclohexane (50 mL) and filtered in hot to extract oxazolidinone **6.31**, which crystallised from cyclohexane as white needles (0.295 g, 32%); mp 119-121 °C (Lit.[17] 122-124 °C); R_f (*n*-hexane/EtOAc, 50:50) = 0.74; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 70:30, 1 $mLmin^{-1}$, λ = 254 nm): t_R = 16.1 and 21.8 min; IR (KBr): ν = 1760, 1710, 1600, 1495, 1390, 1250, 1075

cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.33-6.87 (5H, m, OC_6H_5), 4.95-4.87 (1H, m, OC*H*), 4.37-4.02 (4H, m, CH_2N, CH_2OPh), 2.96 (2H, q, *J* = 7.3 Hz, CH_2CH_3), 1.19 (3H, t, *J* = 7.3 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 174.3 ($NCOCH_2$), 153.1 (O*C*ON), 157.9, 129.8 (2C), 122.1, 114.8 (2C) (aromatic C), 71.8 (O*C*H), 68.1 (CH_2OPh), 44.9 (CH_2N), 29.3 (CH_2CH_3), 8.7 (CH_3); MS (EI+, 70 eV): *m/z* 249 (M^{+}+1, 10%), 107 (12), 94 (29), 77 (20), 57 (100), 28.8 (63); HRMS calcd. for $C_{13}H_{15}NO_4$: 249.1001, found 249.1001; Anal. calcd. for $C_{13}H_{15}NO_4$: C 62.60%, H 6.07%, N 5.62%; found: C 62.1%, H 6.0%, N 5.8%.

Preparation of *N*-(3-hydroxy-2-methylbutanoyl)-5-phenoxymethyl-2-oxazolidinone 6.32[12]

Aldol adducts **6.32** were synthesised following the method previously described for the synthesis of aldol adducts **5.8**, **5.16** and **5.22**. *N*-Propionyl oxazolidinone **6.31** (0.230 g, 0.92 mmol) was reacted with acetaldehyde (260 μL, 4.60 mmol) in the presence of $TiCl_4$ (1 M in DCM, 1.0 mL, 0.97 mmol), (-)-sparteine (210 μL, 0.92 mmol) and NMP (90 μL, 0.93 mmol) to give ***rac-syn*-6.32** (0.084 g, 31%) and other aldol adducts **6.32** (0.045 g, 17%).

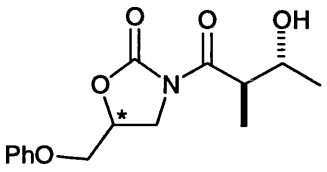
(5*R*,2'*R*,3'*S*)- and (5*S*,2'*S*,3'*R*)-*N*-(3-Hydroxy-2-methylbutanoyl)-5-phenoxymethyl-2-oxazolidinone *rac-syn*-6.32



Alcohol ***rac-syn*-6.32** was obtained as a white solid; mp 119-121 °C; R_f (*n*-hexane/EtOAc, 50:50) = 0.31; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 70:30, 1 $mLmin^{-1}$, λ = 254 nm): t_R = 15.3 and 23.3 min; IR (KBr): ν = 3465, 2915, 1775, 1685, 1380, 1225 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.33-6.86 (5H, m, OC_6H_5), 4.94-4.88 (1H, m, OC*H*), 4.26-4.04 (5H, m, C*H*OH, CH_2N, CH_2OPh), 3.81 (1H, dq, *J* = 2.8 and 7.0 Hz, $COCHCH_3$), 2.90 (1H, s, O*H*), 1.25 (3H, d, *J* = 7.0 Hz, $COCHCH_3$), 1.21 (3H, d, *J* = 6.4 Hz, $CHOHCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 177.2 (N*C*OCH), 152.8 (O*C*ON), 157.8, 129.7 (2C), 121.9, 114.6 (2C) (aromatic C), 71.4 (O*C*H), 67.8 (*C*HOH), 67.7 (CH_2OPh), 44.7 ($COCHCH_3$), 42.9 (CH_2N), 19.5

(CHOH*C*H$_3$), 10.5 (COCH*C*H$_3$); MS (CI+): *m/z* 294 (MH$^+$, 100%), 276 (99), 266 (89), 249 (45), 194 (97), 94 (56), 83 (30); Anal. calcd. for $C_{15}H_{19}NO_5$: C 61.42%, H 6.53%, N 4.78%; found: C 61.5%, H 6.6%, N 4.6%.

Other *N*-(3-hydroxy-2-methylbutanoyl)-5-phenoxymethyl-2-oxazolidinone 6.32



Other *anti* aldol adducts **6.32** were obtained as a white solid; R_f (*n*-hexane/EtOAc, 50:50) = 0.21; HPLC (Chiralcel® OD column, *n*-hexane/isopropanol 70:30, 1 mLmin^{-1}, λ = 254 nm): t_R = 14.2 (8%), 16.3 (85%) and 21.2 (7%) min; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.33-6.81 (5H, m, OC$_6$*H*$_5$), 4.94-4.89 (1H, m, OC*H*), 4.25-4.01 (4H, m, C*H*$_2$N, C*H*$_2$OPh), 3.99-3.93 (1H, dq, app. m, C*H*OH), 3.79 (1H, dq, *J* = 7.0 and 14 Hz, COC*H*CH$_3$), 2.52 (1H, d, *J* = 6.2 Hz, O*H*), 1.24 (3H, d, *J* = 7.0 Hz, COCHC*H*$_3$), 1.21 (3H, d, *J* = 6.2 Hz, CHOHC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 176.4 (N*C*OCH), 152.6 (O*C*ON), 157.5, 129.5 (2C), 121.8, 114.4 (2C) (aromatic C), 71.3 (O*C*H), 70.1 (*C*HOH), 67.7 (*C*H$_2$OPh), 45.2 (CO*C*HCH$_3$), 44.7 (*C*H$_2$N), 21.1 (CHOH*C*H$_3$), 14.8 (COCH*C*H$_3$).

Isolation of *m*-chloroperbenzoic acid

Commercial *m*-chloroperbenzoic acid was freed from *m*-chlorobenzoic acid by dissolving 5.5 g in 150 mL of benzene and washing with an aqueous solution buffered at pH 7.4 (NaH_2PO_4/NaOH, 30 x 2 mL). The organic layer was dried over $MgSO_4$ and carefully concentrated in vacuo. *m*-Chloroperbenzoic acid (5.3 g, 97%) was recrystallysed from DCM and stored at 0 °C.

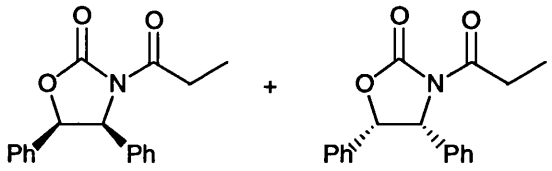
Preparation of t*rans*-stilbene oxide 6.33[21,22]

A solution of *m*-chloroperbenzoic acid (4.7 g, 27.5 mmol) in DCM (200 mL) was added to a solution of *trans*-stilbene (2.5 g, 13.8 mmol) in DCM (300 mL) under an atmosphere of nitrogen. After 20 hours, the reaction mixture was washed with sat. $Na_2S_2O_3$ (400 mL) and H_2O (400 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified *via* flash chromatography (SiO_2, *n*-hexane/EtOAc, 95:5) to give *trans*-stilbene oxide **6.33** (2.3 g, 86%) as white needles; R_f (*n*-hexane/EtOAc, 95:5) = 0.38; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.40-7.15 (10H, m, 2 x C_6H_5), 3.85 (2H, s, 2 x C*H*O); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 137.1, 128.5, 128.3, 128.4, 125.5 (2 x aromatic C), 62.8 (2 x *C*HO). ^1H and ^{13}C NMR data were in accordance with those reported in literature.[22]

General procedure for the preparation of 4,5-diphenyl-*N*-propionyl-2-oxazolidinone *rac-cis* 6.34 and 4,5-diphenyl-2-oxazolidinone *rac-cis*-6.35[20]

Oxazolidinones ***rac-cis*-6.34** and ***rac-cis*-6.35** were synthesised following the procedure above described for the synthesis of *N*-propionyl-5-phenoxymethyl-2-oxazolidinone **6.31**. In a typical experiment, to a solution of **6.30** and **6.33**, DABCO was added and the reaction mixture was refluxed under reduced pressure. For reaction conditions, see Table 6.1. At the end of reaction, the crude brown oil was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc, 70:30) to give either ***rac-cis* 6.34** or ***rac-cis* 6.35** as main product (see Table 6.1 for yields).

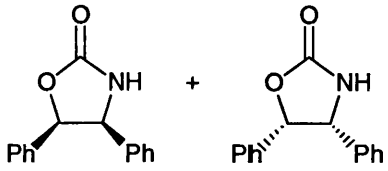
(4*R*,5*S*)- and (4*R*,5*S*)-4,5-Diphenyl-*N*-propionyl-2-oxazolidinone *rac-cis* 6.34



N-Acyl-oxazolidinone ***rac-cis*-6.34** was isolated as a white solid; mp 119-121 °C; R_f (*n*-hexane/EtOAc, 70:30) = 0.52; $[\alpha]_D^{30}$ = 0.0 (*c* 0.9, MeOH); IR (CH_2Cl_2): ν = 1780, 1705, 1345, 1245, 1185 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ_H = 7.12-6.84 (10H, m, 2 x C_6H_5), 5.90 (1H, d, *J* = 7.6 Hz, OC*H*Ph), 5.66 (1H, d, *J* = 7.6 Hz, NC*H*Ph), 3.05 (2H, app. q, *J* = 7 Hz, CH_2CH_3), 1.35 (3H, t, *J* = 7.5 Hz, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C = 173.3 (N*C*OC), 154.0 (O*C*ON), 134.5-125.6 (aromatic C), 80.3 (*C*HO), 62.7 (*C*HN), 29.3 (CH_2), 8.2 (CH_3); MS (CI+): *m/z* 296 (MH^+, 100%), 268 (31), 252

(50), 178 (61), 163 (90), 132 (41), 106.0 (38), 77 (21); Anal. calcd. for $C_{18}H_{17}NO_3$: C 73.20%, H 5.80%, 4.74%; found: C 72.9%, H 6.0%, N 4.9%.

(4*R*,5*S*)- and (4*R*,5*S*)-4,5-Diphenyl-2-oxazolidinone *rac-cis*-6.35[23,24]



Oxazolidinone ***rac-cis* 6.35** was obtained as a white solid; R_f (*n*-hexane/EtOAc, 70:30) = 0.12; $[\alpha]_D^{30}$ = 0.0 (*c* 0.7, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H = 5.18 (1H, d, *J* = 8.1 Hz, NC*H*), 5.35 (1H, s, N*H*), 5.96 (1H, d, *J* = 8.1 Hz, OC*H*), 7.12-6.92 (10H, m, 2 × C_6H_5). ^{1}H NMR data were in accordance with those reported in literature.[23,24]

7.6 References

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Appendix I

X-Ray crystal structure determination of (4*R*,2'*R*,3'*S*)-*N*-(3-hydroxy-2-methylbutanoyl)-4-benzyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.8

k01jmjw1

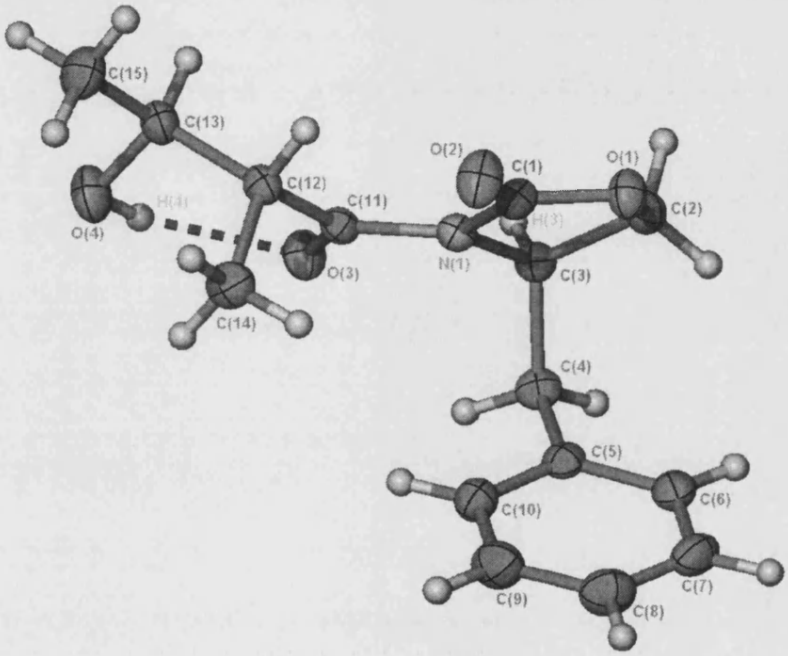

Table 1. Crystal data and structure refinement for 1.

Identification code	k01jmjw1
Empirical formula	C15 H19 N O4
Formula weight	277.31
Temperature	298(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 8.28000(10)Å α = 90°
	b = 12.4340(2)Å β = 90°
	c = 14.2140(3)Å γ = 90°
Volume	1463.38(4) $Å^3$
Z	4
Density (calculated)	1.259 Mg/m^3
Absorption coefficient	0.091 mm^{-1}
F(000)	592
Crystal size	0.50 x 0.40 x 0.35 mm
Theta range for data collection	3.78 to 27.48°
Index ranges	-10<=h<=10; -16<=k<=15; -18<=l<=18
Reflections collected	16756
Independent reflections	3342 [R(int) = 0.0661]
Reflections observed (>2σ)	2534
Data Completeness	0.994
Max. and min. transmission	0.9688 and 0.9558
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3342 / 0 / 189
Goodness-of-fit on F^2	1.024
Final R indices [I>2σ(I)]	R_1 = 0.0359 wR_2 = 0.0785
R indices (all data)	R_1 = 0.0563 wR_2 = 0.0863
Absolute structure parameter	0.0(9)
Largest diff. peak and hole	0.108 and -0.097 $eÅ^{-3}$

Notes: H4 (OH group) located and freely refined.

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 1.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	4749(1)	7184(1)	6858(1)	58(1)
O(2)	5622(2)	8722(1)	7467(1)	69(1)
O(3)	7282(1)	6611(1)	9549(1)	60(1)
O(4)	7910(2)	8080(1)	10926(1)	78(1)
N(1)	6197(1)	7074(1)	8169(1)	41(1)
C(1)	5530(2)	7763(1)	7506(1)	48(1)
C(2)	4709(2)	6068(1)	7124(1)	53(1)
C(3)	5965(2)	5944(1)	7897(1)	46(1)
C(4)	7549(2)	5401(1)	7585(1)	54(1)
C(5)	8447(2)	5981(1)	6819(1)	49(1)
C(6)	8195(2)	5769(1)	5872(1)	61(1)
C(7)	9020(2)	6331(2)	5182(1)	73(1)
C(8)	10128(2)	7086(2)	5418(2)	78(1)
C(9)	10418(2)	7307(2)	6347(1)	75(1)
C(10)	9587(2)	6755(1)	7043(1)	60(1)
C(11)	6808(2)	7341(1)	9046(1)	43(1)
C(12)	6894(2)	8509(1)	9340(1)	48(1)
C(13)	6671(2)	8614(1)	10409(1)	58(1)
C(14)	8474(2)	8995(1)	8974(1)	66(1)
C(15)	6638(3)	9772(2)	10729(1)	80(1)

Table 3. Bond lengths [Å] and angles [°] for 1.

O(1)-C(1)	1.3352(17)	**O(1)-C(2)**	1.4384(18)
O(2)-C(1)	1.1968(17)	**O(3)-C(11)**	1.2198(16)
O(4)-C(13)	1.426(2)	**N(1)-C(11)**	1.3856(18)
N(1)-C(1)	1.3882(17)	**N(1)-C(3)**	1.4704(17)
C(2)-C(3)	1.520(2)	**C(3)-C(4)**	1.540(2)
C(4)-C(5)	1.504(2)	**C(5)-C(10)**	1.385(2)
C(5)-C(6)	1.387(2)	**C(6)-C(7)**	1.385(3)
C(7)-C(8)	1.355(3)	**C(8)-C(9)**	1.370(3)
C(9)-C(10)	1.388(2)	**C(11)-C(12)**	1.513(2)
C(12)-C(14)	1.533(2)	**C(12)-C(13)**	1.536(2)
C(13)-C(15)	1.510(2)		
C(1)-O(1)-C(2)	110.46(11)	**C(11)-N(1)-C(1)**	127.52(12)
C(11)-N(1)-C(3)	120.89(11)	**C(1)-N(1)-C(3)**	111.00(11)
O(2)-C(1)-O(1)	122.45(13)	**O(2)-C(1)-N(1)**	128.35(14)
O(1)-C(1)-N(1)	109.18(12)	**O(1)-C(2)-C(3)**	105.80(11)
N(1)-C(3)-C(2)	100.53(11)	**N(1)-C(3)-C(4)**	112.54(11)
C(2)-C(3)-C(4)	114.78(12)	**C(5)-C(4)-C(3)**	114.80(12)
C(10)-C(5)-C(6)	117.33(16)	**C(10)-C(5)-C(4)**	120.20(15)
C(6)-C(5)-C(4)	122.47(14)	**C(7)-C(6)-C(5)**	121.10(16)
C(8)-C(7)-C(6)	120.54(17)	**C(7)-C(8)-C(9)**	119.82(18)
C(8)-C(9)-C(10)	120.04(18)	**C(5)-C(10)-C(9)**	121.15(16)
O(3)-C(11)-N(1)	117.83(13)	**O(3)-C(11)-C(12)**	122.47(13)
N(1)-C(11)-C(12)	119.69(12)	**C(11)-C(12)-C(14)**	108.95(12)
C(11)-C(12)-C(13)	110.44(12)	**C(14)-C(12)-C(13)**	113.91(13)
O(4)-C(13)-C(15)	107.57(14)	**O(4)-C(13)-C(12)**	112.56(13)
C(15)-C(13)-C(12)	112.41(14)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 \times 10^3$) for 1. The anisotropic displacement factor exponent takes the form: $-2\ gpi^2\ [\ h^2\ a^{*2}\ U11 + ... + 2\ h\ k\ a^*\ b^*\ U$

Atom	U11	U22	U33	U23	U13	U12
O(1)	72(1)	51(1)	51(1)	0(1)	-15(1)	3(1)
O(2)	106(1)	39(1)	62(1)	6(1)	-16(1)	9(1)
O(3)	72(1)	51(1)	57(1)	11(1)	-14(1)	4(1)
O(4)	84(1)	85(1)	64(1)	-2(1)	-28(1)	-2(1)
N(1)	44(1)	35(1)	46(1)	5(1)	-1(1)	2(1)
C(1)	57(1)	41(1)	45(1)	2(1)	0(1)	6(1)
C(2)	54(1)	47(1)	58(1)	-4(1)	-2(1)	-4(1)
C(3)	50(1)	35(1)	53(1)	3(1)	2(1)	-3(1)
C(4)	60(1)	40(1)	62(1)	4(1)	1(1)	7(1)
C(5)	49(1)	43(1)	55(1)	-2(1)	2(1)	12(1)
C(6)	56(1)	61(1)	66(1)	-12(1)	0(1)	10(1)
C(7)	73(1)	95(1)	49(1)	-1(1)	5(1)	22(1)
C(8)	77(1)	87(1)	71(1)	15(1)	15(1)	4(1)
C(9)	69(1)	74(1)	82(1)	1(1)	8(1)	-11(1)
C(10)	60(1)	64(1)	56(1)	-3(1)	1(1)	-3(1)
C(11)	39(1)	45(1)	46(1)	5(1)	0(1)	-2(1)
C(12)	46(1)	46(1)	52(1)	0(1)	-5(1)	-2(1)
C(13)	51(1)	68(1)	54(1)	-5(1)	-7(1)	-6(1)
C(14)	66(1)	55(1)	76(1)	0(1)	8(1)	-13(1)
C(15)	89(1)	77(1)	74(1)	-23(1)	-2(1)	-5(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 1.

Atom	x	y	z	U(eq)
H(2A)	4972	5614	6591	63
H(2B)	3646	5873	7355	63
H(3)	5497	5544	8425	55
H(4A)	8252	5338	8128	65
H(4B)	7304	4679	7369	65
H(6)	7458	5240	5698	73
H(7)	8812	6189	4551	87
H(8)	10689	7452	4951	94
H(9)	11173	7829	6511	90
H(10)	9799	6907	7672	72
H(12)	5999	8889	9036	57
H(13)	5635	8284	10576	69
H(14A)	8580	8845	8314	98
H(14B)	9370	8685	9306	98
H(14C)	8464	9759	9071	98
H(15A)	7636	10115	10561	120
H(15B)	6501	9798	11399	120
H(15C)	5757	10139	10430	120
H(4)	7840(30)	7440(20)	10713(16)	111(9)

Appendix II

X-Ray crystal structure determination of (4*R*,2'*R*,3'*S*)-*N*-(3-hydroxy-2-ethylbutanoyl)-4-benzyl-2-oxazolidinone (4*R*,2'*R*,3'*S*)-5.16

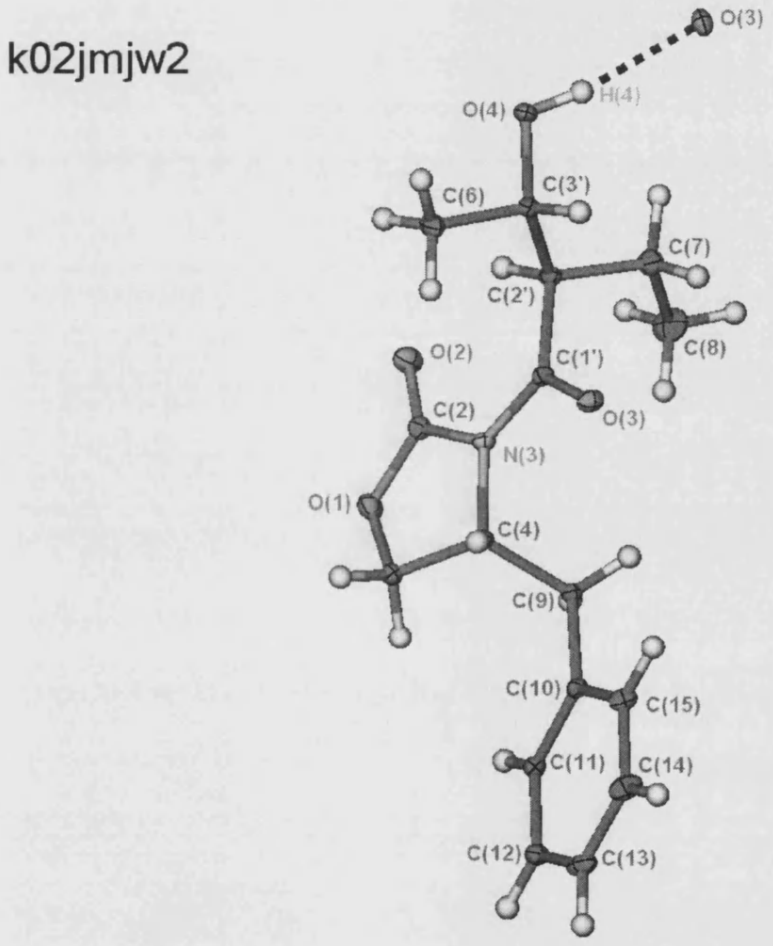

Table 1. Crystal data and structure refinement for 1.

Identification code	k02jmjw2
Empirical formula	C16 H21 N O4
Formula weight	291.34
Temperature	150(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 6.53100(10)Å $\alpha = 90^\circ$
	b = 9.77300(10)Å $\beta = 90^\circ$
	c = 24.2550(3)Å $\gamma = 90^\circ$
Volume	1548.14(3) $Å^3$
Z	4
Density (calculated)	1.250 Mg/m^3
Absorption coefficient	0.090 mm^{-1}
F(000)	624
Crystal size	0.50 x 0.45 x 0.30 mm
Theta range for data collection	3.27 to 30.06 °.
Index ranges	-9<=h<=9; -13<=k<=13; -34<=l<=34
Reflections collected	28745
Independent reflections	4505 [R(int) = 0.0551]
Reflections observed (>2σ)	4210
Data Completeness	0.996
Max. and min. transmission	0.9736 and 0.9566
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4505 / 0 / 192
Goodness-of-fit on F^2	1.067
Final R indices [I>2σ(I)]	$R_1 = 0.0337$ $wR_2 = 0.0824$
R indices (all data)	$R_1 = 0.0380$ $wR_2 = 0.0847$
Absolute structure parameter	0.00
Largest diff. peak and hole	0.240 and -0.252 $eÅ^{-3}$

Notes: Flack parameter not definitive is determining absolute stereochemistry.
H–bonded chains in lattice.

Hydrogen bonds with H..A < r(A) + 2.000 Angstroms and <DHA > 110 deg.

D-H	**d(D-H)**	**d(H..A)**	**<DHA**	**d(D..A)**	**A**
O4-H4	0.840	1.967	170.81	2.799	**O3** [-x+1, y+1/2, -z+3/2]

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for 1.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	3871(1)	1681(1)	9697(1)	30(1)
O(2)	2823(1)	3684(1)	9363(1)	34(1)
O(3)	6225(1)	2271(1)	7994(1)	26(1)
O(4)	1685(1)	5265(1)	7607(1)	29(1)
N(3)	5004(1)	2238(1)	8859(1)	20(1)
C(2)	3790(2)	2651(1)	9306(1)	24(1)
C(4)	6123(2)	963(1)	8986(1)	20(1)
C(5)	4966(2)	486(1)	9501(1)	26(1)
C(6)	1250(2)	2907(1)	7769(1)	33(1)
C(7)	5857(2)	5281(1)	8136(1)	33(1)
C(8)	7102(2)	5546(2)	8659(1)	52(1)
C(9)	8418(2)	1232(1)	9086(1)	21(1)
C(10)	9606(2)	-93(1)	9132(1)	21(1)
C(11)	9874(2)	-743(1)	9641(1)	25(1)
C(12)	10901(2)	-1990(1)	9675(1)	30(1)
C(13)	11687(2)	-2595(1)	9205(1)	33(1)
C(14)	11465(2)	-1949(1)	8700(1)	34(1)
C(15)	10425(2)	-705(1)	8662(1)	28(1)
C(1')	5189(2)	2854(1)	8342(1)	20(1)
C(2')	4169(2)	4208(1)	8217(1)	21(1)
C(3')	2820(2)	4037(1)	7699(1)	22(1)

Table 3. Bond lengths [Å] and angles [°] for 1.

O(1)-C(2)	1.3412(13)	**O(1)-C(5)**	1.4494(13)
O(2)-C(2)	1.1990(13)	**O(3)-C(1')**	1.2222(12)
O(4)-C(3')	1.4279(12)	**N(3)-C(1')**	1.3959(12)
N(3)-C(2)	1.4028(13)	**N(3)-C(4)**	1.4774(12)
C(4)-C(5)	1.5311(14)	**C(4)-C(9)**	1.5408(14)
C(6)-C(3')	1.5165(15)	**C(7)-C(8)**	1.5287(19)
C(7)-C(2')	1.5340(15)	**C(9)-C(10)**	1.5133(14)
C(10)-C(15)	1.3941(15)	**C(10)-C(11)**	1.3993(14)
C(11)-C(12)	1.3938(15)	**C(12)-C(13)**	1.3817(18)
C(13)-C(14)	1.3873(18)	**C(14)-C(15)**	1.3954(16)
C(1')-C(2')	1.5130(13)	**C(2')-C(3')**	1.5429(14)
C(2)-O(1)-C(5)	110.91(8)	**C(1')-N(3)-C(2)**	128.27(8)
C(1')-N(3)-C(4)	120.55(8)	**C(2)-N(3)-C(4)**	111.16(8)
O(2)-C(2)-O(1)	122.37(10)	**O(2)-C(2)-N(3)**	128.94(10)
O(1)-C(2)-N(3)	108.68(9)	**N(3)-C(4)-C(5)**	100.50(8)
N(3)-C(4)-C(9)	111.75(8)	**C(5)-C(4)-C(9)**	113.83(8)
O(1)-C(5)-C(4)	105.44(8)	**C(8)-C(7)-C(2')**	113.15(11)
C(10)-C(9)-C(4)	111.38(8)	**C(15)-C(10)-C(11)**	118.63(10)
C(15)-C(10)-C(9)	120.19(9)	**C(11)-C(10)-C(9)**	121.17(9)
C(12)-C(11)-C(10)	120.52(10)	**C(13)-C(12)-C(11)**	120.37(10)
C(12)-C(13)-C(14)	119.65(10)	**C(13)-C(14)-C(15)**	120.31(11)
C(10)-C(15)-C(14)	120.50(11)	**O(3)-C(1')-N(3)**	117.82(9)
O(3)-C(1')-C(2')	120.83(9)	**N(3)-C(1')-C(2')**	121.34(8)
C(1')-C(2')-C(7)	107.90(9)	**C(1')-C(2')-C(3')**	108.69(8)
C(7)-C(2')-C(3')	112.44(8)	**O(4)-C(3')-C(6)**	106.18(8)
O(4)-C(3')-C(2')	109.38(8)	**C(6)-C(3')-C(2')**	111.98(8)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 \times 10^3$) for 1. The anisotropic displacement factor exponent takes the form: $-2\ gpi^2\ [\ h^2\ a^{*2}\ U11 + ... + 2\ h\ k\ a^*\ b^*\ U$

Atom	U11	U22	U33	U23	U13	U12
O(1)	34(1)	28(1)	27(1)	4(1)	5(1)	2(1)
O(2)	42(1)	31(1)	30(1)	-1(1)	10(1)	12(1)
O(3)	28(1)	28(1)	24(1)	-2(1)	2(1)	8(1)
O(4)	24(1)	26(1)	37(1)	13(1)	-1(1)	2(1)
N(3)	21(1)	18(1)	22(1)	0(1)	-1(1)	4(1)
C(2)	25(1)	26(1)	22(1)	-2(1)	1(1)	0(1)
C(4)	20(1)	17(1)	24(1)	1(1)	-3(1)	2(1)
C(5)	22(1)	22(1)	33(1)	6(1)	0(1)	-1(1)
C(6)	29(1)	30(1)	40(1)	10(1)	-10(1)	-9(1)
C(7)	29(1)	24(1)	47(1)	3(1)	-7(1)	-6(1)
C(8)	45(1)	44(1)	67(1)	-8(1)	-21(1)	-14(1)
C(9)	19(1)	19(1)	27(1)	2(1)	-1(1)	0(1)
C(10)	17(1)	20(1)	26(1)	2(1)	-3(1)	-1(1)
C(11)	20(1)	26(1)	29(1)	5(1)	0(1)	1(1)
C(12)	24(1)	26(1)	40(1)	11(1)	-3(1)	0(1)
C(13)	27(1)	21(1)	50(1)	-2(1)	-11(1)	4(1)
C(14)	32(1)	34(1)	38(1)	-12(1)	-8(1)	9(1)
C(15)	27(1)	31(1)	28(1)	-2(1)	-5(1)	6(1)
C(1')	19(1)	19(1)	21(1)	-2(1)	-2(1)	1(1)
C(2')	21(1)	18(1)	25(1)	1(1)	-1(1)	2(1)
C(3')	21(1)	21(1)	24(1)	5(1)	-1(1)	0(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for 1.

Atom	x	y	z	U(eq)
H(4)	2431	5840	7446	35
H(1)	5942	286	8681	24
H(5A)	3999	-259	9407	31
H(5B)	5932	151	9784	31
H(6A)	419	2836	7433	49
H(6B)	363	3117	8083	49
H(6C)	1954	2036	7834	49
H(7A)	6793	4969	7841	40
H(7B)	5225	6150	8014	40
H(8A)	8149	6240	8584	78
H(8B)	7765	4695	8777	78
H(8C)	6191	5873	8952	78
H(9A)	8976	1781	8778	26
H(9B)	8588	1766	9430	26
H(11)	9351	-330	9967	30
H(12)	11061	-2426	10022	36
H(13)	12375	-3449	9229	39
H(14)	12024	-2355	8377	41
H(15)	10274	-272	8314	34
H(2')	3288	4480	8535	25
H(3')	3703	3840	7371	26

Appendix III

CAL B activity assay

Lipase type B derived from *Candida antarctica* (Chirazyme® L-2, carrier-fixed, Carrier 3, lyophilizate from Boehringer Mannheim) was tested, prior to use, for both hydrolytic and transesterification activity.

Hydrolytic activity

Lipases in nature hydrolyse tributyrin to dibutyrin, monobutyrin and glycerol. The activity assay measures the liberation of butyric acid by titration with sodium hydroxide. One lipase Unit is defined as the amount of enzyme that liberates one micromole of titrable butyric acid per minute at pH 7.0, with tributyrin as substrate.

Procedure: 40 mL 10 mM phosphate buffer, pH 7.0, was incubated in a thermostated vessel at 25 °C, equipped with a magnetic stirrer. After addition of tributyrin (1.16 mL, 1 mmol), CAL B (40 mg) was added.
The pH was checked and kept constant at 7.0 with an automated pH-stat system (Mettler DL21 Titrator, NaOH 1M). The consumption of 1 M NaOH was monitored for 20 minutes. The specific activity was calculated for the base consumption at the linear part of the resulting graph. The same assay was performed without enzyme and considered as a control.
In 20 min 3.125 mmol of NaOH 1M were consumed, which means that 3.125 $x10^3$ mmol of tributyrin were converted by the enzyme

Units = (mmol substrate converted) /min = 156.25 U

40 mg of the used enzyme preparation contained 156.25 lipase Units, which correspond to an activity of 3.9 U/mg $_{\text{dry form}}$.

Transesterification activity

Lipases are well known to catalyse transesterification reactions, if the reaction is performed in organic solvents such as *n*-hexane, toluene, dichloromethane, methyl ter-butyl ether, and tertiary alcohols. The reaction substrate is generally a secondary alcohol. Vinyl esters or anhydrides are used as acylating agents.

Procedure: Organic solvent (10 mL *n*-heptane), vinyl acetate (275 ml, 3 mmol) and substrate (140 ml 1-phenylethanol, 1 mmol) were filled into the reaction vessel and stirred until the substrate was dissolved. CAL B was added and the suspension was stirred for 48 hours.

Conversion and enantioselectivity were followed by chiral HPLC (Chiralcel® OJ column, *n*-hexane/isoproanol 99:1, 1 $mLmin^{-1}$, λ = 254 nm). After 48 hours, the percentage of conversion was 61% and the enantiomeric excess was >99%.